



## Efficacy of inactivated BEF vaccine adjuvant with Montanide ISA 206 compared with the locally prepared inactivated BEF vaccines.

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### ABSTRACT

Bovine ephemeral fever (BEF) virus belonging to Ephemerovirus genus of the Rhabdoviridae family causes an economically important seasonal acute vector-borne disease. Routine vaccination, is one of the main controlling steps, particularly in countries where the disease is endemic. This study deal with comparative evaluation of humeral immune response of cattle using SNT and ELISA to three inactivated BEF vaccines. Inactivated BEF vaccine with Montanide ISA 206 (water-in-oil-in-water) adjuvant given in 2 doses one month apart gave high protective antibody titer lasted for 44 weeks' post vaccination (WPV). The inactivated BEF vaccine with aluminum hydroxide gel adjuvant given in 2 doses one month apart gave satisfactory immune response lasted for 30 WPV then neutralizing antibodies waned rapidly. Live attenuated BEF vaccine that inactivated by saponin on time of vaccination give higher antibody titer which rise more rapidly lasted for 38 WPV only. It could be concluding that the inactivated BEF vaccine with Montanide ISA 206 adjuvant gave satisfactory higher and longer lasting antibody titer for 44 WPV and it was recommended to use this vaccine in endemic and non-endemic countries to control and prevent the disease especially before summer season.

**KEYWORDS:** BEF virus, Vaccine, Adjuvant, SNT, ELISA.

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### 1. INTRODUCTION

Bovine ephemeral fever (BEF) virus belonged to genus Ephemerovirus of the family Rhabdoviridae, has been isolated from mosquitoes and Culicoides biting midges and causes an economically important seasonal acute vector borne disease (Kirland, 1995; Mellor, 1996). BEF disease is characterized by biphasic fever, depression, lameness, recumbency, joint swelling, rear limb paralysis, nasal and ocular discharge, excessive salivation, stiffness, sudden drop in milk production and spontaneous recovery in three days. The disease spreads through subtropical and temperate regions of the Middle East, Asia, Africa, and Australia (Finlaison et al., 2010). BEF virus possesses a single stranded, negative-sense RNA genome and five structural proteins comprising a nucleoprotein (N), a polymerase-associated protein (P), a matrix protein (M), a large RNA-dependent RNA polymerase (L) and a glycoprotein (G) spanning the viral envelope (Walker et al., 1991). A glycoprotein (G) is a type-specific neutralizing antigen and induces protective immunity in cattle (Uren et al., 1994), the N protein is also immunogenic in cattle but it does not induce virus-neutralizing antibodies or a protective response, it induces a T cell proliferative response in cattle (Uren et al., 1993). Control of BEF disease depend mainly

on, restricted cattle movement, a brief quarantine period in a vector-free area beside vector control, treatment and vaccination. To date four types of BEF vaccine have been developed live attenuated, inactivated, G protein-based sub-unit and recombinant vaccines but the most commercially used types are live attenuated and inactivated vaccines (Walker and Klement, 2015). Live attenuated BEF vaccine have been used in many endemic countries that produced a long-lasting neutralizing antibody (Tzipori and Spradbrow, 1973), but their use was discouraged owing to their potential lack of safety because the virus might back-mutate to its virulent form (Muskett et al., 1985). Inactivated BEF vaccine adsorbed on aluminum phosphate gel (Inaba et al., 1973) and water-in-oil-in-water adjuvant (Aziz-Boaron et al., 2013) were likewise utilized. In Egypt, binary ethylene imine inactivated BEF vaccine with aluminum hydroxide gel adjuvant and attenuated BEF vaccine inactivated on the time of vaccination using saponin were applied in the field (El-Behwar et al., 2010). Although experimental and commercial BEF vaccines have been developed in different formulations, there is a need for further researches to

provide a more informed evaluation of the vaccine performance (Walker and Klement, 2015).

In this study an inactivated BEF vaccine was prepared using Montanide ISA 206 (water-in-oil-in-water) as adjuvant, examined for sterility and safety then evaluated for humeral immune response in calves through follow up of antibody titers for 48 weeks using SNT and ELISA, in comparison to other locally prepared inactivated gel adjuvant BEF vaccines.

## 2. MATERIAL AND METHODS

### 2.1. 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 and used as the seed for vaccine preparation after its adaptation to tissue culture.

### 2.2. BEF Vaccines:

#### 2.2.1. Local inactivated BEF vaccine with aluminum hydroxide gel as adjuvante:

It was supplied by the Department of Pet Animal Vaccine Research, Veterinary Serum and Vaccine Research, Institute, Abbassia, Cairo ( $10^8$  tissue culture adapted virus vaccine).

#### 2.2.2. Local live attenuated BEF vaccine inactivated on time of vaccination using saponin:

It was supplied by the Department of Pet Animal Vaccine Research, Veterinary Serum and Vaccine Research, Institute, Abbassia, Cairo ( $10^8$  tissue culture adapted virus vaccine).

### 2.3. Saponin:

It was supplied under the cat. no. 16109; lot. 71500 by sigma-Aldrich Labochemikalien Gm6H; Germany. It was supplied as 0.2  $\mu\text{g}$  saponin/ml with phosphate buffered saline (PBS) and was used as a diluent to inactivate the live attenuate BEF virus vaccine at the time of vaccination and injected directly after reconstitution.

### 2.4. Cell line:

Baby Hamster Kidney cell line (BHK<sub>21</sub> clone 13) was supplied by the Animal Research Institute, Pirbright, UK. It was propagated at FMD Department, Veterinary Serum and Vaccine Research, Institute, Abbassia, Cairo, Egypt by using of minimum Essential Medium (MEM) with Eagle's salts and with 10 % new born calf serum according to (Macpherson and Stocker, 1962). These cells

were used for virus propagation and titration and also for SNT

### 2.5. Vaccine formulation:

BEF virus was inoculated on BHK-21 cell line that harvested at over 70% cytopathic effect after 30–35 h, 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  $10^8$ TCID<sub>50</sub>/ml and 64 using infectivity titration and CFT, respectively. It was inactivated by binary ethylene imine (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).

### 2.6. 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 10x dose of the vaccine in calves by subcutaneous (S/C) route.

### 2.7. Calves and experimental design:

Nineteen 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 in comparison to other local BEF vaccines as follow: Group (1): Five calves vaccinated with 2ml/animal of the prepared oil vaccine by subcutaneous (S/C) route and boosted after one month. Group (2): Five calves vaccinated with 2ml/animal (S/C) of the local inactivated BEF vaccine adjuvant with aluminum hydroxide gel and boosted after one month. Group (3): Five calves vaccinated (S/C) with the local live attenuated lyophilized BEF vaccine reconstituted in PBS containing 0.2  $\mu\text{g}$  saponin/ml used as a diluent to inactivated the virus on time of vaccination. Group (4): Two calves were kept as control without vaccination. Group (5): Two calves were used in safety test of the prepared vaccine. Sera from

vaccinated cattle groups were used to measure the variance in efficacy and duration of immunity between different BEF vaccines for 48 weeks, using SNT and ELISA test.

**2.8. 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<sub>50</sub> of BEF virus (Mellor, 2001).

**2.9. 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 gave satisfactory results being free from aerobic and anaerobic bacteria and fungi. The prepared vaccine was safe and gave

satisfactory results after tested on tissue culture with no appearance of cytopathic effect, and no rise in body temperature of vaccinated calves group (4).

**3.2. Immunological response:**

For calves in group (1), SNT results showed that protective neutralizing serum antibody titer (1.2) started from 2<sup>nd</sup> WPV, reached highest titer at 12<sup>th</sup> WPV and persisted in protective titer till 44<sup>th</sup> WPV, as shown in table (1) and figure (1); ELISA results showed that positive serum antibody level started from 2<sup>nd</sup> WPV, reached highest level at 12<sup>th</sup> WPV and persisted positive till 48<sup>th</sup> WPV, as shown in table (2) and figure (2).

For calves in group (2), SNT results showed that protective neutralizing serum antibody titer (1.2) started from 2<sup>nd</sup> WPV, reached highest titer at 10<sup>th</sup> WPV and persisted in protective titer till 30<sup>th</sup> WPV, as shown in table (1) and figure (1); ELISA results showed that positive serum antibody level started from 2<sup>nd</sup> WPV, reached highest level at 10<sup>th</sup> WPV and persisted positive till 30<sup>th</sup> WPV, as shown in table (2) and figure (2).

Table (1): Mean serum neutralizing antibody titers for BEF virus in sera from calves vaccinated with different types of BEF vaccines using SNT.

Weeks post vaccination	Mean log <sub>10</sub> serum neutralizing antibody titers			
	Group 1	Group 2	Group 3	Control
1	*0.75	0.86	0.9	0
2	1.35	1.35	1.47	0
3	1.65	1.47	1.89	0
4	1.98	1.67	2.07	0
6	2.4	1.86	2.4	0
8	2.52	2.07	2.76	0
10	2.61	2.34	2.67	0
12	2.7	2.13	2.58	0
14	2.58	2.04	2.34	0
16	2.43	1.97	2.19	0
18	2.34	1.85	2.04	0
20	2.07	1.76	1.92	0
22	2.01	1.61	1.79	0
24	1.89	1.53	1.78	0
26	1.83	1.41	1.55	0
28	1.74	1.36	1.47	0
30	1.62	1.26	1.38	0
32	1.56	1.12	1.33	0
34	1.5	1.08	1.25	0
38	1.41	0.93	1.21	0
40	1.29	0.72	0.69	0
44	1.23	0.3	0.33	0
48	0.99	0	0.12	0

Protective serum neutralizing antibody titer=1.2

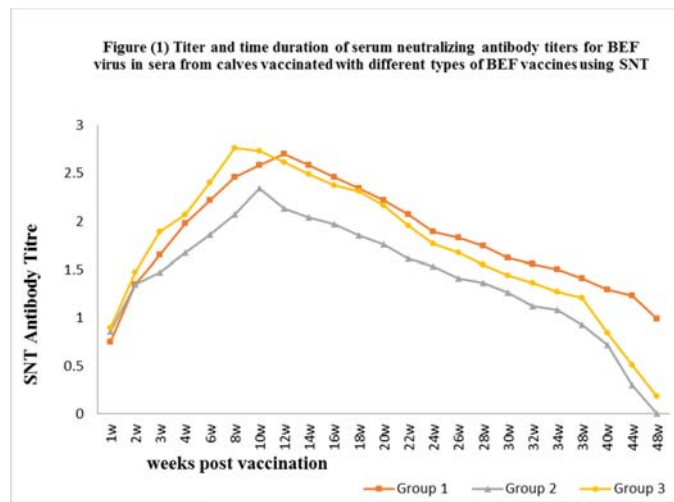


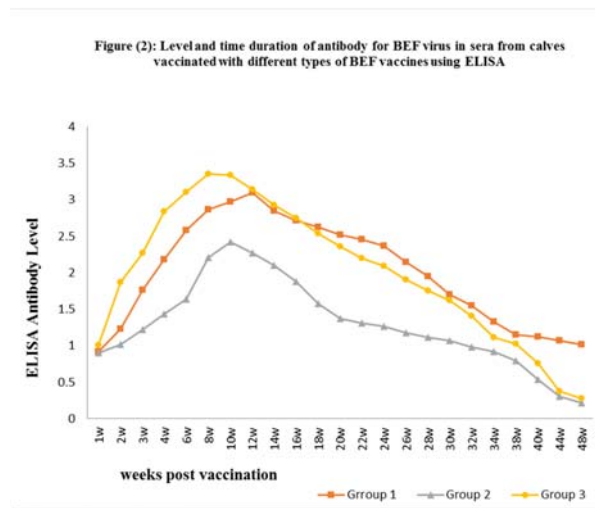
Table (2): Mean serum antibody levels for BEF virus in sera from calves vaccinated with different types of BEF vaccines using ELISA

Weeks post vaccination	Mean optical density for sera of vaccinated calves using ELISA			
	Group 1	Group 2	Group 3	Control
1	0.9194	0.9034	1.0066	0.220
2	1.2256	1.0178	1.8654	0.214
3	1.7614	1.2194	2.2666	0.204
4	2.1826	1.4364	2.8362	0.215
6	2.5752	1.637	3.1028	0.198
8	2.8658	2.2024	3.354	0.213
10	2.9676	2.4176	3.3308	0.224
12	3.0928	2.2686	3.136	0.223
14	2.8436	2.0976	2.9294	0.196
16	2.713	1.8758	2.7508	0.198
18	2.6256	1.5736	2.5348	0.213
20	2.5186	1.3676	2.3608	0.190
22	2.4582	1.305	2.1958	0.210
24	2.3678	1.2642	2.0948	0.193
26	2.1476	1.1744	1.9032	0.196
28	1.9446	1.1162	1.7538	0.204
30	1.6974	1.0724	1.6204	0.190
32	1.545	0.9846	1.4028	0.202
34	1.3288	0.9172	1.114	0.197
38	1.1504	0.796	1.0228	0.203
40	1.1222	0.5356	0.762	0.193
44	1.067	0.305	0.373	0.194
48	1.016	0.2142	0.2824	0.198

Positive serum antibody level over one.

For calves in group (3), SNT results showed that protective neutralizing serum antibody titer (1.2) started from 2<sup>nd</sup> WPV, reached highest titer at 8<sup>th</sup> WPV and persisted in protective titer till 38<sup>th</sup> Weeks PV, as shown in table (1) and figure (1); ELISA results showed that positive serum antibody

level started from first WPV, reached highest level at 8<sup>th</sup> WPV and persisted positive till 38<sup>th</sup> WPV, as shown in table (2) and figure (2). These results were shown in comparison to that of the calves kept as control non-vaccinated Group (3) that gave negative results.



#### 4. DISSCUSSION

In Egypt, many outbreaks of BEF were reported native and imported breeds of cattle (Al-Gaabary et al., 2005 ; Davies et al., 1993; El-Bagoury et al., 2014; Hassan, 2000; Kawther and Wahid, 2011). Routine vaccination, as one of the main controlling steps, is a critical tool in controlling BEF disease, particularly in countries where the disease is endemic. This study deal with comparative evaluation of humeral immune response of cattle to three types of BEF vaccines including inactivated vaccine with ISA 206 oil adjuvant, inactivated vaccine with aluminum gel adjuvant and live attenuated vaccine inactivated at time of vaccination using saponin. Testing sterility of the prepared inactivated BEF vaccine with ISA 206 oil adjuvant showed its freedom of foreign contaminants (bacteria and fungi) (Code of Federal Regulation of USA, 1986).

The prepared inactivated BEF vaccine proved to be safe in tissue culture with satisfactory results with no cytopathic effect, and it gave also satisfactory results of safety in calves with no rise in body temperature and no clinical abnormalities (Henderson, 1970). For calves in group (1) inoculated with the prepared inactivated BEF vaccine, protective neutralizing serum antibody titer (1.35) started from 2<sup>nd</sup> WPV, reached highest titer at 12<sup>th</sup> WPV and persisted in protective titer (1.23) till 44<sup>th</sup> WPV using SNT while ELISA results showed that positive serum antibody level started from 2<sup>nd</sup> WPV, reached highest level at 12<sup>th</sup> WPV and persisted positive (1.016) till 48<sup>th</sup> WPV. It was recorded that vaccine prepared from low and high passage BEF virus emulsified with Freund's incomplete mineral oil adjuvant could induce high neutralizing antibodies in vaccinated cattle which could be detectable for at least a year (Theodoridis

et al., 1973), especially in case of boosting that gave longer antibody response and higher titer than single injection (Cameron et al., 1987). Vaccines adjuvanted with Montanide ISA 206 can promote longer lasting immunity than conventional vaccines adjuvant with Alhydragel in ruminant persisted for at least 6 months PV (Barnett et al., 1996; Hunter, 1996).

For calves in group (2) inoculated with the local inactivated BEF vaccine with aluminum hydroxide gel adjuvant, protective neutralizing serum antibody titer (1.35) started from 2<sup>nd</sup> WPV, reached highest titer at 10<sup>th</sup> WPV and persisted in protective titer (1.26) till 30<sup>th</sup> WPV, using SNT while ELISA results showed that positive serum antibody level (1.0178) started from 2<sup>nd</sup> WPV, reached highest level at 10<sup>th</sup> WPV and persisted positive (1.0724) till 30<sup>th</sup> WPV. Formalin inactivated, aluminum phosphate gel-adsorbed vaccine developed in Japan was shown to elicit a strong antibody response after two doses but immunity waned rapidly and neutralizing antibody was no longer detected in most animals (Inaba et al., 1973), and it was found also that oil emulsion FMD vaccine (double oil emulsion) gave best results in comparison with AL (OH)<sub>3</sub> vaccine (Barteling and Vreswij, 1991).

Antiviral activity of saponin was studied in vitro against some viruses where it was found to inhibit the replication of herpes simplex virus type 1(HSV-1) and poliovirus type 2 as shown by inhibition of cytopathic effect and reduction of virus production. In addition, saponin action involves inhibition of virus-host cell attachment and interferes with both early and late events of virus replication (Amoros et al., 1987). Also its effect might be the cause of inhibition of virus penetration and protein synthesis (Hayashi et al., 1997).

For calves in group (3) inoculated with live attenuated BEF Virus vaccine which inactivated by saponin at time of vaccination, SNT results showed that protective neutralizing serum antibody titer (1.47) started from 2<sup>nd</sup> WPV, reached highest titer at 8<sup>th</sup> WPV and persisted in protective titer (1.21) till 38<sup>th</sup> WPV, using SNT while ELISA results showed that positive serum antibody level (1.006) started from first WPV, reached highest level at 8<sup>th</sup> WPV and persisted positive (1.0228) till 38<sup>th</sup> WPV. These results agreed with those obtained by Black (1997) who indicated that saponin was a more efficient adjuvant than aluminum hydroxide gel where antibodies are higher when saponin was used. Also Samuikenko et al. (1982) found that saponin in an aluminum hydroxide gel, formaldehyde treated FMD vaccine raised the protection and neutralizing antibody titers. Although live attenuated BEF vaccine inactivated by saponin on time of vaccination gave rapid rise of higher antibody titer persisted for 38<sup>th</sup> WPV that recommended to use in case of outbreaks to control the disease but inactivated BEF vaccine with Montanide ISA206 adjuvant gave longer duration of immunity. The inactivated BEF vaccine with aluminum hydroxide gel adjuvant gave satisfactory immune response for 30<sup>th</sup> WPV but it waned rapidly and neutralizing antibody was no longer detected as in case of other two tested vaccines.

Finally, we can conclude that the inactivated BEF vaccine with Montanide ISA 206 (water-in-oil-in-water) adjuvant gave higher and longer duration antibody titer for 44<sup>th</sup> WPV after two vaccinations one month apart, that it was recommended to use this vaccine in endemic and non-endemic countries to control and prevent the disease especially before summer season.

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