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Comparative evaluation of three formulae of bovine ephemeral fever virus vaccines

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ABSTRACT

Bovine ephemeral fever virus (BEFV) is an arthropod-borne virus. BEFV causes a debilitating disease in cattle and water buffaloes. It is enzootic in bovine populations in Egypt causing great economic losses in animal wealth. Vaccination against BEFV is an important factor for controlling the disease. Many studies were applied to improve the locally produced vaccine using different adjuvants in formulation of BEF vaccine. This study aimed to prepare and evaluate potency of an inactivated BEF vaccine with Montanide™ Gel-01 adjuvant using serum neutralization test and ELISA. The pattern of humeral immune response to the prepared vaccine was compared with that of the local inactivated BEF vaccine with aluminum hydroxide gel adjuvant and live attenuated BEF vaccine inactivated just before inoculation by saponin. The obtained results revealed that best vaccine was 20% Montanide Gel 01™ inactivated BEF vaccine, As it give higher antibody titer throughout the experiment that extend for more than one year post vaccination compared with that of other vaccinated groups, followed by BEF vaccine inactivated with saponin and 15% Montanide™ Gel-01 based BEF vaccine . Whereas, aluminum hydroxide gel; 5% and 10% Montanide™ Gel-01were poor inducer for humeral immune response. So 20% Montanide™ Gel-01based inactivated BEF vaccine could be recommended in BEF vaccination program.

1. INTRODUCTION

Bovine ephemeral fever virus (BEFV) is a negative-stranded RNA virus genus Ephemerovirus, family Rhabdoviridae. It is an arthropod born virus that transmitted by many species of biting midges and mosquitoes (Walker and Klement 2015). BEFV has serious economic impacts through cessation of milk production and loss of condition of cattle and water buffalo; besides subclinical involvement of a variety of ruminant species (Walker, 2005). The disease is prevalent throughout Africa, the Middle East, the Indian Sub-continent, South and South-East Asia, the Russian Federation, Japan and Australia—but not, as yet, in America or Europe (except western parts of Turkey) (Walker and Klement 2015). A lot of epizootics with BEFV had been found in Egypt (Hassan, 2000; Daoud et. al., 2005; Younis et. al., 2005, Nayel, 2006; Kasem et.al., 2014; and Albehwar et. al.,2018). So, efficient vaccination is essential for disease control. The earlier BEF vaccines were prepared from BEFV field isolates that attenuated by serial passaging in suckling mice and or cell culture (Van der Westhuizen, 1967). Various adjuvants such as Freund's complete or incomplete adjuvant, aluminum hydroxide, dextran sulfate, or Quil A were used for inactivated BEF vaccines (Tzipori and Spradbrow; 1973; Vanselow et. al., 1995). However; live vaccines create long lasting neutralizing antibodies that could be presented for 12 months or more prior to vaccinations, they were prone to be impaired by heat or light or they could cause adverse clinical reactions (Tzipori and Spradbrow 1973; Tzipori and Spradbrow 1978). Whereas; inactivated vaccines are safer as the ability of the pathogen to propagate in the vaccinated host is destroyed while the viral capsid remains intact, so it is still recognizable by the immune system. Inactivation of BEFV has been achieved using a variety of agents such as formalin (Inaba et. al.; 1973), β-propiolactone (Della-Porta and Snowdon, 1979) and binary ethyleneimine (Hsieh etal., 2006; Daoud et al, 2001; Saber, 2004 and Albehwar et al, 2010, El-Bagoury et al, 2016). Consequently, selection of proper adjuvant is one of vaccine production progress that increases humoral and cell-mediated immune response, as the convenient adjuvant could elaborate high and long-standing immunity (Dalsgrrd et al., 1990). Our study aims to promote and enhance the immunogenicity of the locally produced BEF vaccine to induce high and long duration of humeral immunity in vaccinated animals using Montanide Gel 01™ as an adjuvant.

2. MATERIAL AND METHODS

The experiment was ethically approved under the following number BUFVTM01-05-21

2.1. Virus strain and cell line

Baby Hamster Kidney (BHK) cell culture adapted Local strain BEFV (BEFV/Abbasia/2000). It was supplied by VSVRI. The virus titer was $10^{7.5}$ TCID50/ml (Azab, et al., 2002). It was used in propagation of BEFV for vaccine formulation. BHK cell lines were grown and maintained according to Macpherson and stocker (1962), and was used

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for propagation and titration of BEFV and serum neutralization test (SNT)

2.2. Montanide™ Gel-01

Montanide™ Gel-01 is a water-based adjuvant designed to improve the efficacy of aqueous type vaccines. It is an aqueous dispersion of a synthetic polymer classified in the category of high molecular weight polyacrylic acid. MontonideTM Gel -01 could be adjusted between 5, 10, 15 and 20% (W/W). It was obtained from SEPPIC, France (SEPPIC, 2008).

2.3. Vaccines

Locally prepared lyophilized live BEF vaccine "strain BEF/Abassia/2000" (Batch No.1/2019) which was inactivated on the time of vaccination using its specific saponin diluent (Albehwar et al, 2010) [was supplied by VSVRI and inactivated BEF vaccine with aluminum hydroxide gel (Daoud et al, 2001)]. They were used for vaccination of Calves for comparative evaluation with the prepared inactivated BEF vaccine with Montanide™Gel-01adjuvant.

2.4. Preparation of inactivated BEF vaccine with Montanide-01 gel

BEFV was propagated on BHK cells, titrated and the virus titre was adjusted to $10^{7.5}$ TCID50/ml, then inactivated using 3% of 0.01M Binary Ethyleneimine (BEI) for 4 hours at 37° C prepared (Girard et. al., 1977 and Daoud et. al., 2001). Samples from the inactivated virus were checked for the presence of active virus in BHK cells according to OIE (2012).

The inactivated virus fluid was mixed with different concentrations (5%, 10%, 15% and 20%) of the Montanide-01 gel as adjuvant, using magnetic stirrer (SEPPIC, 2008). The prepared vaccines were tested for their freedom from Mycoplasma, aerobic, and anaerobic bacteria, fungi (FAO, 1994).

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 was applied by injection 2 x doses of the vaccine in calves by subcutaneous (S/C) route.

2.6. Calves vaccination studying safety and Comparative evaluation of different BEF vaccine formulae

One year Thirty-five cross breed calves were used for studying safety and humeral immune response of the prepared BEF vaccines. All of these animals were screened using SNT proved to be free from BEFV antibodies. They were vaccinated with 2 doses with 2 weeks interval divided by injection of 2ml different BEF vaccine formulae s/c in the neck side. They were divided into4 groups as follows: -

Group 1: It was composed of 5 calves. They were vaccinated with attenuated cell culture BEF vaccine inactivated on the time of use (Albehwar et. al., 2010).

Group 2: It included 5 calves vaccinated with inactivated BEF vaccine with aluminium hydroxide adjuvant (Daoud et al., 2001).

Group3: it was 5 calves which were vaccinated with 5% Mantonide-01 gel inactivated BEF vaccine.

Group4: it consisted of 5 calves vaccinated with 10% Mantonide-01 gel inactivated BEF vaccine.

Group5: it was 5 calves which were vaccinated with 15% Mantonide-01 gel inactivated BEF vaccine.

Group6: five calves were vaccinated with 20% Mantonide-01 gel inactivated BEF vaccine.

Group 7: it was 5 calves were kept without vaccination as control negative.

The vaccine safety was evaluated after first vaccination. The Rectal temperature was examined daily from the day of vaccination until 10 days. Moreover, the injection area was inspected to detect local reactions

2.7. Serum samples:

Serum samples were collected from calves before and after vaccination weekly (4 times), then monthly up to 12 months post vaccination with the different BEF vaccine formulae. Sera were stored at –20ºC and inactivated at 56ºCfor 30 minutes before being used in SNT and ELISA.

2.8. Serum Neutralization Test (SNT):

It was used to detect and quantify BEF neutralizing antibodies in the serum of vaccinated calves according to (Rossiter, et. al., 1985). Briefly, serial double fold dilutions of vaccinated calves' serum were pre incubated with constant titre of BEFV for sufficient time for neutralization prior to inoculation on to BHK-21 cells. Detection of neutralizing antibodies was determined by lack of cytopathic effect on BHK-21cells. The neutralizing antibody titre was estimated according to Singh et al., (1967).

2.9. Enzyme Linked Immune Sorbent Assay (ELISA):

Bovine ephemeral fever virus antigens from infected BHK-21 cells were immobilized to a 96-well polystyrene microtiter plates by passive absorption (Wagner et al., 1969), then used in in direct ELISA to determine specific BEF antibodies (Lin, 2015). The results were expressed as optical density that was depending on amount of antibody in tested sera.

3. RESULTS

Satisfactory results were obtained after testing of inactivated BEFV strain on tissue culture with no appearance of cytopathic effect. All vaccine preparations were free from any bacterial, fungal or mycoplasmal growth during the period of observation (14 days) as revealed by sterility test. The vaccine safety was evaluated as no any local reaction at site of injection was observed Moreover; no significant difference was observed between rectal temperatures measured in the vaccinated and control groups in addition to; no undesirable signs or conditions were observed for 10 days

3.1. Results of serum neutralization test (SNT):

The humeral immune response of vaccinated calve serum against different BEFV vaccine formulae were showing great variation as shown in table (1). The highest antibodies titer with the longest duration were induced by BEF vaccine with 20% Mantonide-01 gel as 256 Neutralization unit (NU) by2nd month post the second dose followed by live attenuated cell culture BEF vaccine saponin inactivated on the time of use as 128. The immune response induced by inactive BEF vaccine adjuvanted with 15% Montanide™Gel-01 was similar to live attenuated BEF vaccine saponin inactivated on the time of use till 10th month post booster vaccination. All of the pervious

formulation showed prolonged immunity duration up to 12 month. While as; the aluminum hydroxide, Montanide™Gel-01 (5%-10%) adjuvanated-vaccines were poor inducer for antibody production as, lower neutralization unit were recorded as 64; 64 and 32 for aluminum hydroxide gel; 10 and 5% Montanide™Gel-01 respectively with shorter immunity duration (9 months). While, the protective level of BEF antibodies were produced after one week post second dose of vaccine administration. It was 32 neutralizing unit forBEF vaccine saponin inactivated, and inactive BEF vaccine with 15% Montanide™Gel-01 while the inactive BEF vaccine with 20% Montanide™Gel-01 neutralizing unit was 64 that was exceed than the protective level.

3.2. Results of Enzyme linked immunosorbant assay (ELISA):

Results of ELISA came in a parallel manner with that of SNT confirming each other, showing the optical density of montanide gel 20% based BEF vaccine is the highest one followed by saponine based one. They were the long lasting vaccines in their immune response. While ELISA values for aluminium hydroxide gel; 10 and 5% Montanide™Gel-01adjuvanted BEF vaccine were low as shown in table (2) and fig (1).

Table 1 Mean BEF serum neutralizing antibody titers induced by different BEF vaccine formulae

Animal group	Days after 1st vaccination				Mean BEF serum neutralizing antibody titer days after booster dose													
	0	7	14		7 th DPB	14 th DPB	1 th MPB	2 th MPB	3 th MPB	4 th MPB	5 th MPB	6 th MPB	7 th MPB	8 th MPB	9 th MPB	10 th MPB	11 th MPB	12 th MPB
G1	0	8	16	booster	32	64	128	128	128	128	128	128	128	128	128	128	64	32
G2	0	4	8		16	32	64	64	64	64	64	64	64	64	32	32	16	8
G3	0	2≤	2		4	8	16	32	32	32	32	32	16	16	8	8	4	2
G4	0	2	4		8	16	32	64	64	64	64	64	64	32	16	16	8	4
G5	0	8	16		32	64	128	128	128	128	128	128	128	128	128	128	128	64
G6	0	8	16		64	128	256	256	256	256	256	256	256	256	256	256	256	128
G7	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	0

Six groups of were vaccinated with BEF vaccine formulae as follow, G(1) live attenuated BEF vaccine inactivated on the time of use,G(2) inactivated BEF vaccine with aluminium hydroxide gel,G(3) inactivated BEF vaccine with 5% Mantonide-01 gel,G(4) inactivated BEF vaccine with 10% Mantonide-01 gel,G(5) inactivated BEF vaccine with 15% Mantonide-01 gel,G(6) inactivated BEF vaccine with 20% Mantonide-01 gel,G(7) control un vaccinated group MPB: month post boostering,DPB : days post boostering

Table 2 Mean values of ELISA optical density in calves vaccinated with different BEF vaccine formulae

Animal group	Days after 1st vaccination				Mean values of ELISA optical density days and months after booster dose													
	0	7	14		7 th DPB	14 th DPB	1 th MPB	2 th MPB	3 th MPB	4 th MPB	5 th MPB	6 th MPB	7 th MPB	8 th MPB	9 th MPB	10 th MPB	11 th MPB	12 th MPB
G1	0.22	1.0	1.8	Booster	2.18	2.83	3.10	3.45	3.50	3.53	3.50	3.50	3.48	2.92	2.50	2.00	1.90	1.00
G2	0.24	1.0	1.4		1.60	2.20	2.45	2.50	2.50	2.53	2.54	2.50	2.50	2.20	1.87	1.57	1.30	0.90
G3	0.04	0.4	0.7		1.50	1.78	1.80	2.11	2.11	2.01	2.22	2.01	2.11	1.87	1.50	0.91	0.53	0.35
G4	0.03	0.5	0.9		2.10	2.11	2.40	2.43	2.43	2.41	2.45	2.41	2.43	2.00	1.50	1.11	0.98	0.79
G5	0.02	0.5	1.7		2.43	2.44	2.50	3.00	3.11	3.11	3.00	3.11	3.00	2.50	2.43	1.80	1.40	1.17
G6	0.28	0.5	1.9		2.50	2.55	2.58	3.50	3.50	3.53	3.45	3.53	3.50	2.90	2.75	2.50	1.90	1.50
G7	0.21	0.2	0.1		0.22	0.19	0.20	0.22	0.19	0.21	0.23	0.21	0.19	0.22	0.21	0.19	0.19	0.19

Positive ELISA titer was over 1, six groups of were vaccinated with BEF vaccine formulae as follow, G(1) live attenuated BEF vaccine inactivated on the time of use,G(2) inactivated BEF vaccine with aluminium hydroxide gel,G(3) inactivated BEF vaccine with 5% Mantonide-01 gel,G(4) inactivated BEF vaccine with 10% Mantonide-01 gel,G(5) inactivated BEF vaccine with 15% Mantonide-01 gel,G(6) inactivated BEF vaccine with 20% Mantonide-01 gel,G(7) control un vaccinated group MPB: month post boostering,DPB : days post boostering.

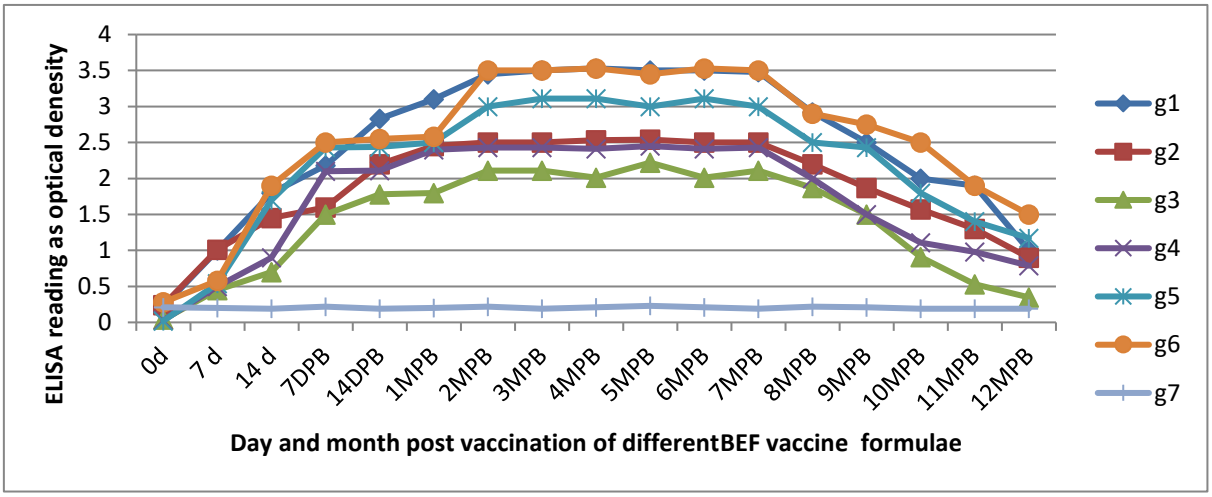

Fig (1) the curves of immune response based on ELISA. G(1) live attenuated BEF vaccine inactivated on the time of use,G(2) inactivated BEF vaccine with aluminium hydroxide gel,G(3) inactivated BEF vaccine with 5% Mantonide-01 gel,G(4) inactivated BEF vaccine with 10% Mantonide-01 gel,G(5) inactivated BEF vaccine with 15% Mantonide-01 gel,G(6) inactivated BEF vaccine with 20% Mantonide-01 gel,G(7) control un vaccinated group MPB: month post boostering,DPB : days post boostering

4. DISCUSSION

Since BEFV is enzootic in bovine populations in Egypt, control efforts are mainly directed to an efficient vaccination of susceptible animals. The vaccine production improvement is directed towards the selection of the proper adjuvant that might influence the vaccine properties crucially and elaborate high and long-lasting immunity. Adjuvant is an ingredient used in vaccine formulation that helps to create a stronger immune response. So, in our study, we have used a series of well-characterized adjuvants to stimulate the immune response in calves against BEF vaccine. The range of adjuvants included aluminum hydroxide, which is one of the most widely used adjuvant in practical vaccination (Lind blad, 1995), as well as montanide™ gel -01 adjuvant which was previously reported to be good stimulators of humeral and cell-mediated immunity. Montanide™ Gel - 01 is an adjuvant that gives sufficient early immune response in vaccinated cattle (Dupuis et. al., 2006). Montanide™ Gel-01 has already been used in several vaccine models with a promising safety and efficacy (Parker et. al., 2009). It is simple to formulate, formed stable emulsion and of stability at least one year at 4ºC (AbulMagd et. al., 2014a). Montanide™ Gel-01 clearly retards the elimination of antigens thus permit immune response to last longer in the form of sustained release antigen, so the immune response being antigen driven respond to the presence of antigen (Tizard, 2009). More over; It Enhances phagocytosis of the antigen complex with the polymer therefore raising the activity of antigen presenting cells (Vialle et. al., 2010). Montanide™ gel-01is a good intensifier for cell mediated immune response as indicated by phagocytic activity, gamma interferon and interleukin-6 responses and induced protection against challenge with Newcastle and Avian Influenza (H9N2) viruses (El-Naggar et. al., 2017). Furthermore; the highest possible protective antibody titer as observed in Montanide™ Gel -01 based pneumonic pasteurellosis vaccine in cattle (Mogahid, 2019). Our results revealed that 20% of Montanide™ Gel-01 enhanced the immune response of BEF vaccinated calves providing them with the highest and long lasting antibody titers as shown in table (1 and 2) and fig (1). This result was agreeable to (Abd El Rahman et. al,2020, AbulMagd et. al., 2014a and El-Sayed et. al., 2011). As they proved thatMontanide™ Gel -01 at concentration 20% in preparation of inactivated RVF vaccine to get a better control of the disease among animals. Similar results were recorded by (Uren et. al., 1994; Daoud et. al., 2001; Mostafa, 2004; Khalid, 2004; Saber, 2004; Daoud et. al., 2005 and Younis et.al., 2005) who showed that vaccination of cattle with 2 doses of the inactivated BEF vaccine was capable to induce high levels of immunityagainst BEF virus infection. In addition to, live attenuated BEF vaccine in activated on time of administration and BEF in activated based Montanide™ Gel -01 at concentration 15 %vaccine were somewhat similar to each other in their humeral immune response. They were good inducer for humeral immune response. It is acceptable as Albehwar et al (2010) stated that the use of live BEF vaccine which was inactivated on the time of administration induced high levels of specific BEF neutralizing antibodies where the diluent of such vaccine contains saponin which act as virus inactivator and immune stimulant.it might be attributed to in complete adsorption of BEF antigen to Montanide™ Gel -01 at concentration 15 % as observed in the adsorption assay that made byAbd El Rahman et al, 2020, where the complete adsorption for RVF antigen occurred withMontanide™ Gel -01 at concentration 20%.Although the aluminum hydroxide gel based BEF vaccine and BEF vaccines with 10% and 5% Montanide™ Gel -01 were poor immune stimulatory adjuvants . These results were similar to El-Sayedet. al., 2011asan intenseimmune response was observed with Montanide™ Gel -01 20% with prolong antibody secretion in comparison to the aluminum hydroxide. Moreover; Trials for Montanide™ Gel -01 based vaccine in cattle declared that post vaccinal reaction was entirely absent other than using aluminum-based formulations that induce light pyrogenic reaction (Tizard, 2009).

5. CONCLUSION

Our data will contribute in BEF vaccine progress as the obtained result conclude that, 20% Montanide Gel 01TM was highly immunogenic as it accelerates, potentiate and prolong immune response when used as adjuvant in preparation of BEF, so we recommend it to be used in animal vaccination to increase the level and duration of immune response. Further study is required to determine the in-need revaccination.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

6. REFERENCES

1. Abd El Rahman ,S.E.;AbulMagd, D. M.;Atwa, M. H., Soliman, S. M.(2020): Evaluation of The Cellular and Humoral Immune Response of Sheep Vaccinated with Inactivated Rift Valley Fever Vaccine Adjuvanted with Montanide Gel 01™ Journal of Applied Veterinary Sciences, vol.5(1):22-34.
2. Albehwar, A.M.; Nermein, G. Shafeik; Saad, M.A.; Ibrahim, M.M.; Magda, S. Mohamed; AnharAbd El-Moety and Khodeir, M.H. (2010): Comparative evaluation of the potency of traditionally inactivated bovine ephemeral fever vaccine and that one inactivated on the time of vaccination; 14th Sci. Cong.2010, Fac. Vet. Med. Assiut Univ. Egypt: 51-61.
3. Albehwar, A.M.; Wafaa, M. Ismaeil; Hiam, M. Fakhry and Hemmat, S. El-Emam (2018): Molecular characterization of recent isolates of BEF virus in Egypt; Global J. Med. Res. Vet. Sci. Vet. Med. Vol. 18 Issue (2): 6-14.
4. AbulMagd D.M.; Ebied M.; Abdel Wahab M.G.; El-Sayed M.; Galila E.M.; Moustafa A.M. and Sayed E.M. (2014a): Evaluation of humeral immune response in sheep vaccinated with Monanide™Gel (01) adjuvanted Rift Valley Fever vaccine, Minufiya Vet. Med. J. Vol. 8 (1): 263-273 Special Issue for the 8th Sci. Conf. Fac. Vet. Med. Univ. Sadat City
5. Azab, A.M.H.; Khodier, M.H.; Attyat,M.Kotb and El-Gallad, S.B. (2002): Susceptibility of different cell cultures to bovine ephemeral fever virus; 6th Vet. Med. Zag. Conf.: 41-55.
6. Dalsgrrd, K.; Hilgers,L. and Trouve,G. (1990):Classical and new approache to adjuvant use in domestic animals. Adv. Vet. Sci.Comp. Med., 35: 121-159.
7. Daoud, A.M.; Soad, M.Soliman; Azab, A.M. and Taha, M.M. (2001): Preparation of inactivated bovine ephemeral fever vaccine (BEF) in Egypt; Beni-Suef Vet. Med. J., Vol. XI, No. (2): 619-626.
8. Daoud, A.M.; Younis, E.E.; El-Sawalhy, A.A.; Sayed-Ahmed, M.Z.S. and Khodier, M.H. (2005): Some studies on bovine ephemeral fever in buffaloes. 4th Inter.Sci.Conf.Mansoura, April 2005: 687-701.
9. Della-Porta, A.J. and Snowdon, W.A. (1979): An Experimental Inactivated Virus Vaccine against Bovine Ephemeral Fever: 2- Do Neutralizing Antibodies Protect Against Infection? Veterinary Microbiology 4: 197-208.

10. Dupuis L.; Ascarateil S.; Aucouturier J. and Ganne V. (2006): SEPPIC vaccine adjuvants for poultry, Annals of the New York Academy of Sciences 1081, 202-205.
11. El-Bagoury, G.F.; El-Habbaa, A.S; Amal, A.M.; Nermeen, G.S (2016):Efficacy of inactivated BEF vaccine adjuvant with Montanide ISA 206 compared with the locally prepared inactivated BEF vaccines. Benha veterinary medical journal, 31, no. (2):117-123.
12. El-Naggar H.M.; Madkour M.S. and Hussein H.A. (2017): Preparation of mucosal nanoparticles and polymer-based inactivated vaccine for Newcastle disease and H9N2 AI viruses, Vet. World, 10 (2): 187-193
13. El-Sayed E.L.; Abdel-Hady M.K.; Zaki F.S.A.; Mahmoud M.S.; Ahmed I.K. and Ghaly H.M. (2011): Study on improvement of Entero-3 vaccine using Monanide^{TM}Gel (01) as an adjuvant, Proc.4th Sci. Conf. Anim. Wealth Res. In Middle East and North Africa, foreign agriculture relations (FAR), Egypt, 551-559.
14. FAO (1994): Quality control of rinderpest cell culture vaccine. Standard Operating Procedures, FAO report No.118.
15. Girard,H.C.; Bayramoglu,O.; Erol.,N. and Burgut,A. (1977): "Inactivation of (O1) FMD virus by binary ethylenemine (BEI)" Bull. Off. Int. Epiz. 87(3-4): 201-217.
16. Hassan, H.A. (2000): An outbreak of bovine ephemeral fever in Egypt during 2000: II-Clinical and haematological alteration before and after symptomatic treatment trials. 9thScin.Cof. Fac. Vet. Med. Assiut Univ. Egypt.
17. Hsieh, Y.C.; Wang, S.Y.; Lee, Y.F.; Chen, S.H. and Mak, P.O. et al. (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. Journal Veterinary Medicine Science, 68: 543-548.
18. Inaba, Y.; Kurogi, H.; Sat, K.; Goto, Y. and Omori, T. (1973): Formalin-inactivated, aluminum phosphate gel-adsorbed vaccine of bovine ephemeral fever virus.ArchGesamteVirusforsch, 42: 42-53.
19. Kasem, S.;Dawod, A.;Khodier, M.;Kotb, A.;abouelyazeed, M.; Abdel Kareem, S. and Ibrahim, B. (2014): Isolation and Characterization of Bovine Ephemeral Fever Virus in Delta Provinces, Egypt 2012. Global Veterinarian 13 (6): 972-976.
20. Khalid, S. M. (2004):Some studies on bovine ephemeral fever in cattle. M.V. Sci. Thesis (Infectious Diseases) Fac. Vet. Med. Zagazig Univ., Benha Branch, Moshtohor.
21. Lin A.V. (2015): Indirect ELISA. In: Hnasko R. (eds) ELISA. Methods in Molecular Biology, vol 1318. Humana Press, New York, NY.
22. Lindblad, E. B. (1995):Aluminum adjuvants, p. 21–35. In D. E. S. Stewart-Tull (ed.), The theory and practical application of adjuvants. John Wiley & Sons, New York, N.Y.
23. Mogahid, B.E. (2019): Some immunological and antigenic studies on pneumonic pasteurellosis in cattle, Ph. D. Thesis (Bacteriology, Immunology and Mycology) Fac. Vet. Med. Benha Univ.
24. Mostafa, M. A. (2004):Epidemiological studies on bovine ephemeral fever in Egypt Ph.D. Vet. Sci. Thesis (Infectious diseases) Fac. Vet. Med. Cairo Univ.
25. Mackpherson, L.A. and Stocker, M.G.P. (1962): Polyoma transformation of hamster cell colonies; Annvestigation of genetic factors affecting cell competence; Virology, 16: 147.
26. Nayel, M.A. (2006):Serosurvey of bovine ephemeral fever in Menoufia Governorate, M.V.Sci.Thesis (Infectious diseases); Fac. Vet. Med. Menoufia University.
27. OIE ,2012. OIE Terrestrial manual 2012, chapter 2.1.14.
28. Parker R.; Deville S.; Dupuis L.; Bertrand F. and Aucouturier J. (2009): Adjuvant formulation for veterinary vaccines: Monanide^{TM}Gel (01) safety profile; ProcediaVaccinology, Vol.1, Issue (1), page: 141-147.
29. Rabagliati, D. S. (1924): "Three days' fever or sickness in cattle" Vet. Rec. 4: 503-5.
30. Rossiter, P.B.; Jesset, D.M. and Taylor, W.P. (1985):Microneutralization system for use with different strains of pest des petit ruminant's virus and rinderpest virus. Trop. Anim. Heal. Prod., 17 (2): 75-81.
31. Saber, S.M.M.(2004): Epidemiological studies on ephemeral fever in cattle.;Ph. D. Vet. Sci. Thesis (Infectious diseases) Fac. Vet. Med. Cairo Univ
32. Seppic (2008): Technical Bulletin Montanide TM01 gel ready to use aqueous polymeric adjuvant for veterinary vaccines.
33. Singh, K.V.; Osman, O.A.; Thanaa, I.Baz and Ivon, El-Cicy (1967): Colostral transfer of rinderpest neutralizing antibodies to offspring of vaccinated dams. Can. J. Comp. Med. Vet. Sci., 31: 295-298.
34. Tizard Ian R. (2009): Veterinary Immunology and Introduction, 8th Ed, Elsevier, USA Sanders, CuritsCenter, Philadelphia 127: 49-55
35. Tzipori, S. and Spradbrow, P. B. (1973): Studies on vaccines against Bovine-Ephemeral Fever. Aust. Vet J 49: 183-187.
36. Tzipori, S. and Spradbrow, P.B. (1978): A cell culture vaccine against bovine ephemeral fever.Aust. Vet. J., 54: 323-328.
37. Uren, M.F.; Walker, P.J.; Zakrzewski,H.; St-George, T.D. and Byrne, K.A. (1994): Effective vaccination of cattle using the virus as an antigen. Vaccine, 12 (9): 845-850.
38. Van der Westhuizen, B. (1967): Studies on bovine ephemeral fever: I-Isolation and preliminary characterization of a virus from natural and experimentally produced cases of bovine ephemeral fever. Onderstepoort J. Vet. Res., 34: 29-40.
39. Vanselow, B.A.; Walthall, J.C. and Abetz,I. (1995): Field trials of ephemeral fever vaccines in Queens's land. Vet.Microbiol.23: 117-130.
40. Vialle R.; Dupuis, L.; Deville, S.; Bertand F.; Gaucheronand J. and Aucouturier J. (2010): Microgel particulate adjuvant: Characterization and mechanisms of action, ProcediaVaccinology 2 (2010): 12-16.
41. 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.
42. Walker, P.J. (2005): Bovine ephemeral fever in Australia and the world. Curr Top Microbiol Immunol.;292:57-80. doi: 10.1007/3-540-27485-5_4. PMID: 15981468.
43. Walker, P.J. and Klement, E. (2015): Epidemiology and control of bovine ephemeral fever. Vet Res 46, 124–142.
44. Younis, E.E.; Sayed-Ahmed, M.Z.; Khodier, M.H.; El-Sawalhy, A.A. and Daoud, A.M. (2005): Studies on bovine ephemeral fever in cattle. 8th Sci. Cong. Egyp.Soci. Cattle Dis., Assuit, Egypt: 4-13.