Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine
Volume 18 Issue 2 Version 1.0 Year 2018
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Enhanced Immune Responses of Trivalent Foot and Mouth Disease Vaccine using Montanide Oil and Clinoptilolite Adjuvants in Cattle

By Hiam M. Fakhry, Assem A. Mohamed, Ekbal M Farouk, Hegazi, A. Z. & Namaa A. Mohamed

University Veterinary Serum and Vaccine

Abstract- Adjuvant play an important role in the efficacy of vaccines, the protective immune response produced by vaccines can vary according to the kinds of adjuvant. The comprehensive sero-immunological study was conducted to reveal the adjutant's effect of Clinoptilolite and oil on the immune response of trivalent Foot and mouth disease (FMD) vaccine in cattle. This study was conducted in five cattle groups; The first group was vaccinated intramuscularly (I/M) with trivalent FMD Clinoptilolite (1 **µ**g/dose) vaccine, The second group was vaccinated with FMD (Oil + Clinoptilolite) vaccine and Third group was vaccinated with FMD oil vaccine while the fourth group were non vaccinated used as negative control and fifth group were used for safety test. Then conducted tests to compare the enhancement in cattle immunity. The humeral and cellular immune responses were monitored in different tested groups.

Keywords: FMD virus, vaccine, clinoptilolite, XTT, SNT, and ELISA.

GJMR-G Classification: NLMC Code: QW 70

Strictly as per the compliance and regulations of:

Enhanced Immune Responses of Trivalent Foot and Mouth Disease Vaccine using Montanide Oil and Clinoptilolite Adjuvants in Cattle

Hiam M. Fakhry [α], Assem A. Mohamed [σ], Ekbal M Farouk [ρ], Hegazi, A.Z. [ω] & Namaa A. Mohamed [¥]

Abstract- Adjuvant play an important role in the efficacy of vaccines, the protective immune response produced by vaccines can vary according to the kinds of adjuvant. The comprehensive sero-immunological study was conducted to reveal the adjutant's effect of Clinoptilolite and oil on the immune response of trivalent Foot and mouth disease (FMD) vaccine in cattle. This study was conducted in five cattle groups; The first group was vaccinated intramuscularly (I/M) with trivalent FMD Clinoptilolite (1 µg/dose) vaccine, The second group was vaccinated with FMD (Oil + Clinoptilolite) vaccine and Third group was vaccinated with FMD oil vaccine while the fourth group were non vaccinated used as negative control and fifth group were used for safety test. Then conducted tests to compare the enhancement in cattle immunity. The humeral and cellular immune responses were monitored in different tested groups. The obtained results indicated that the incorporation of Clinoptilolite into inactivated FMD vaccine induces an increase of the specific protective immune response. Higher and longer period of immune responses were found in cattle vaccinated with both Montanide oil and Clinoptilolite adjuvanted vaccine up to 40 weeks, while those vaccinated with Clinoptilolite or oil vaccine showed protected immunity up to 32 weeks respectively. Finally, we recommended that using of Clinoptilolite with oil as a potential adjuvant in FMD vaccine.

Keywords: FMD virus, vaccine, clinoptilolite, XTT, SNT, and ELISA.

I. Introduction

Foot-and-mouth disease (FMD) is an acute infectious disease that infects cloven-hoofed mammals, such as pigs, cattle, cattle and goats (Dar et al., 2013). The causative agent is a single-stranded positive- sense RNA virus that belongs to the genus Aphthovirus in the family Picornaviridae. The virus has seven serological types, identified as; O, A, C, SAT1, SAT2, SAT3 and Asia1 (Dar et al., 2013).

FMD is characterized by fever, lameness and vesicular lesions on the feet, tongue, snout, and teats, with high morbidity and low mortality (Rodriguez and Grubman 2009).

In Egypt, the disease is enzootic, and outbreaks have been reported since 1950, Type O was the most prevalent since1960 (Zahran 1960, Farag et al., 2005 And Satya 2009). FMDV serotype A was isolated during 2006 in Egypt through live animals importation where sever clinical signs were recorded among cattle and buffaloes Abed El-Rahman (2006). Also FMDV serotype SAT2 was recorded in Egypt (Shawky et al., 2013 and Nader et al., 2014).

Control of FMD in animals was considered to be important to effectively contain the disease in endemic areas, so that vaccination is effective in limiting the spread of FMD (Depa et al., (2012).

The vaccine adjuvant is the very important factor which stimulates specific components of either cellular or humeral immune response Lombard (2007), Fakhry et al., (2012) and Sonia et al., (2015). Most foot-and-mouth disease vaccines are made of BEI (binary Ethyleneinmine) inactivated virus that is adjuvanted with oil adjuvant.

The in-house produced vaccine by Veterinary Serum and vaccine Research Institute (VSVRI) is the Montanide ISA 206 trivalent inactivated vaccine which Consists of three FMDV serotypes (O/ Pan Asia1, A Iran/05 and SAT2/EGY?2012.

Adjuvants, also can prolong the immune response and stimulate specific components of the immune response either humeral or cell-mediated (Lombard et al., 2007).Continuous improvement of formulations to obtain the highly immunogenic vaccine, The improvement not only depend on the antigen payload, but also selecting the ideal or the most suitable adjuvant is one of the important tools in improving the efficacy of the FMD vaccine. Adjuvant is one which can stimulate the humeral immune response early (onset), and promote the production of high antibody titers that would long duration. It should also stimulate the cellular immune response (Park 2013).

The oil adjuvant has the capability for generating a rapid, high and long-lasting immune response. Generally, the Montanide

Series of oil adjuvant (SEPPIC, France) has a immunological effect for inactivated vaccine in different susceptible animals (Fakhry et al., 2012, Dar et al., 2013, and Ehab et al., 2015).

Clinoptiolite is a natural, non-toxic that has monoclinic crystal structure symmetry (Mansouri et al 2013). Also Clinoptiolite not classified as to their carcinogenicity to humans and animal (Dong et al.,

Author α σ ρ ω ¥: Veterinary Serum and Vaccine Research Institute (VSVRI), Department of FMD, Abbasia, Cairo, Egypt P. O. Box 131, e-mails: svri@idsc.gov.eg, dr_hfakhryalhawary@yahoo.com

2003). Clinoptiolite has been extensively tested for toxicity in a wide range of animals, including rats, mice, hamsters, beagles, and pigs appear to lack toxic effects unless ingested in very large quantities (European Parliament 1997), it does not have any side effect (Ray Sahelian 2016).

Clinoptilolite is a micro mineral particle that in earlier studies has shown adjuvant activity against different antigens. Clinoptilolite is safe and effective (Garces 1999 and Rhodes2010). Clinoptilolites play an important role in regulating the immune system. (Aikoh et al., 1998) have reported that silica, silicates, and aluminosilicates act as nonspecific immunostimulators similarly to super antigens. Super antigens are a class of immunostimulatory and disease-causing proteins of bacterial and viral origin with the ability to activate relatively large fractions (5-20%) of the T cell population, as well as humoral immune responses.

The purpose of this study was to evaluate the efficacy of Clinoptilolites in addition to ISA 206 as an adjuvant of inactivated trivalent FMDV, to stimulate the immune response.

II. Materials and Methods

a) Animals

i. *Cattle*

21 cattle were clinically healthy and free from antibodies against FMDV.

ii. *Unweaned baby mice*

30 Swiss Albino suckling mice (three to five days old were) classified into six groups, used in safety test of inactivated virus and vaccines and supplied by the Lab. animal's farm of Veterinary Serum and Vaccine Research Institue, Abbasia, Cairo, Egypt.

b) FMD virus Strains

Local FMDV strains (O /pan Asia2, A/ Iran 05 and SAT2/ Egypt 2012) were isolated and identified by Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. and confirmed by Pirbright (FMD-WRL), United Kingdom. FMDV were propagated in BHK21 cell line in roller bottles (Huang et al., 2011), each virus had an infectivity titer of 108 TCID50/ml as described by (Reed and Muench 1938). These viruses were used as virus mitogens in the lymphocyte proliferation assay, vaccine preparation and SNT

c) Inactivation of FMD virus

FMD virus strains were inactivated with mixture of 1 mM binary ethyl eneimine (BEI) and 0.04% formaldehyde according to the method described by (Sarkar et al., 2017) sodium this sulfate 20% was added to the virus samples to the inactivated virus to neutralize the BEI in a final concentration of 2%. Sodium bisulfite 20% was added after inactivation process to neutralize the excess of formalin in final concentration of 2%.

d) Adjuvants

i. *Montanoid Oil*

ISA 206 Montanide Oil was obtained from Seppic, Paris, France.

ii. *Clinoptilolite*

The fine powder of natural clinoptilolite was obtained by Micronisiertes Klinoptilolith – Hochwertigs Naturminera, Germany.

e) Formulation of the prepared vaccines

i. *Vaccine 1: Clinoptilolites adjuvant vaccine*

Trivalent inactivated FMD with 1 μg/doses of Clinoptilolites according to (Mansouri et al., 2013, and (Hiam and Assem 2014).

ii. *Vaccine 2: Oil and Clinoptilolites adjuvant vaccine*

Trivalent inactivated FMD with Montanide ISA 206 +1μg/doses of Clinoptilolites according to (Alhawary et al., 2017)

iii. *Vaccine 3*

Trivalent inactivated FMD with Montanide ISA 206 according to (Barnett et al., 1999).

f) Evaluation of the prepared vaccine formulations: Sterility and safety testing

The vaccines were cultured on Sabouraud's, nutrient agar; thioglycolate broth, phenol dextrose media and mycoplasma medium. The tested vaccines were free from any aerobic, anaerobic bacteria and fungal contaminants. The Safety of inactivated virus and vaccines were done according to (OIE 2013).

i. *Evaluation of Cellular Immunity*

Heparinized blood samples were obtained from vaccinated and control non- vaccinated animals at 0, 3, 7, 14, 21, 28, 35 and 42 days post vaccination.

Stimulation of the cellular immune response by the different prepared FMD vaccine was evaluated using cell proliferation kit (XTT kit) according to EL-Naggar (2012).

ii. *Evaluation of humeral immune response of vaccinated animals*

Serum samples were collected from the vaccinated and non-vaccinated cattle weekly post-vaccination for one month then every 2 weeks post vaccination up to 40 weeks for evaluation of antibody titers against FMDV strains (O /pan Asia2, A/Iran 05 and SAT2/Egypt 2012) in serum samples were measured using the neutralization assay as described previously (OIE 2012) and indirect ELISA according to (Voller et al., 1976).

g) Experimental Design

21 cattle were classified into five groups, five animals for each first three groups. The first group was vaccinated with 3 ml intramuscularly (I/M) with trivalent FMD Clinoptilolite (1 μg/dose) vaccine, the second group was vaccinated with 3 ml FMD

(oil + clinoptilolite) vaccine and Third group was vaccinated with 3ml FMD oil vaccine. While the fourth group (three animal) were none vaccinated used as negative control and fifth group (three animal) were used for safety test.

III. Results and Discussion

Foot and Mouth Disease (FMD) is an acute disease caused by Foot and Mouth Disease Virus (FMDV) which causes economy losses (Orsel et al., 2007). In endemic areas the vaccination of animals is effective in control and limiting the spread of FMD.

FMD vaccines can be defined as a specific formulation of chemically inactivated virus strains and mix with a suitable adjuvant.

Selecting the suitable vaccine formulation is dependent on several factors as the onset of protection and the duration of protection against FMD.

The effective formulation of inactivated FMD vaccines requires adjuvant Clinoptilolite, and Montanide ISA 206 mineral oil-based formulations have been widely employed in experimental studies to obtain a vaccine that stimulates a rapid and long-lasting protective immune response, the formulated vaccines are safe for animal use.

In this work, we studied the effect of natural Clinoptilolite particles to induce specific and protective immune response against foot and mouth disease.

The formulation Clinoptilolites-FMDV is non toxic with adjuvant activity (Batista et al., 2010). Vaccine formulations containing the adjuvant could promote the presentation of the virus so it could increase the immune response and the protection (Batista, et al., 2010 and Fakhry et al., 2012).

Stimulation of the cellular immune response by the different prepared FMD vaccine was evaluated using Lymphocyte blastogenesis using XTT assay) according to (Scudiero et al., 1988).

The obtained results of cell-mediated immune response using lymphocyte proliferation test for all animal groups expressed by ΔOD (Delta Optical Density) were as follow: 3 ml intramuscularly (I/M) with trivalent FMD Clinoptilolite (1 µg/dose) vaccine. The second group was vaccinated with 3 ml FMD (oil + Clinoptilolite) vaccine and Third group was vaccinated with 3ml FMD oil vaccine.

In group 1 (trivalent FMD Clinoptilolite vaccine): Delta Optical Density was (0.517) by using FMD viruses at 3rd -day post vaccination(DPV) and still rise reached its highest level (1.557) at 3rd -week post vaccination(WPV) and continue high within examination time 35 DPV.

In group 2 (trivalent FMD oil + Clinoptilolite vaccine: Delta Optical Density was (0.515) by using FMD viruses at 3rd –DPV and still rise reached its highest level (1.665) at 2nd – WPV, and continue high within 35 DPV then declined.

In group 3 (trivalent FMD oil vaccine): Delta Optical Density was (0.473) by using FMD viruses at 3rd - DPV and still rise reached its highest level (1.136) at 3rd - WPV then declined gradually as shown in Table No. (1).

From Tables (1) showed the results of cell- mediated immune response using lymphocyte proliferation test for all animal groups expressed by ΔOD (Delta Optical Density) appeared to be supported by (Sharma et al., 1984) they reported that cell mediated immune response was a constitute of immune response against FMD virus, and in agreement in some points with (Mercedes et al., 1996, El-Watany et al., 1999, Sonia et al., 2010 and El-Din, W et al., 2014) whose found that FMD vaccine stimulated the cellular immune response and lymphocyte stimulation by FMDV was greater than by mitogens (PHA) and appeared the highest increase in 1st and 2nd -WPV, while disagreed with (El-Watany et al., 1999). The obtained results were in agreement with (des 2010) who mentioned that ClinoptiloDavid 2013), our results also were supported by (Rholite enhance cell mediated I mmune response.

a) Tracing the antibody titer against FMDV serotypes (O, A&SAT2)

The SNT and ELISA data (Tables2&3) show differences in the onset, intensity and duration of the FMD serotype O, A &SAT2 antibodies elicited by the different vaccine formulations. Concerning the onset of protection, it is clear that FMD Clinoptilolite vaccine (group1 and FMD Clinoptilolite + oil vaccine (group3) reach the protective level at 2nd WPV early than group (2) FMD oil vaccine which reach protective level at 3rd WPV The results revealed that SNT titers for FMD vaccines, go in hand with the results obtained are consistent with the statement of (Wisniewski et al., 1972) they explained that the SNT measures those antibodies which neutralize the infectivity of FMD virion. The peak of antibody titre in all groups at 10-12 WPV and continues with protective level till 32th WPV in FMD Clinoptilolite vaccine and FMD oil vaccine groups while in FMD Clinoptilolite+ oil vaccine group till 40th WPV. The results agreed with (Kreimir et al., 2000, and Rhodes 2010) who showed that adjuvant properties of Clinoptilolite as potent adjuvant induced higher antibody titers than the antigen alone or vaccine adjuvanted with Montanide oil and improved the potency of adjuvants. Results supported also by (Batista et al., 2010) they found that Clinoptilolite help the vaccine work more effectively, increasing antibody production. Who found that Clinoptilolite might help the vaccine work more effectively, increasing antibody production, also Clinoptilolite improved B-cells function, improved mucosal and humoral immunity and protective activity also helped vaccine for induction strong immunity when used as adjuvant. Our results also go in hand with the results obtained were consistent with the statement of

(Hamblin et al., 1986) who explained that the SNT measures those antibodies which neutralize the infectivity of FMD virion, while ELISA probably measure all classes of antibodies even those produced against incomplete and non-infectious virus.

Finally, it can conclude that: The usage of Clinoptilolite as an adjuvant alone or preferable with ISA 206 oil in inactivated FMD trivalent vaccine induces long lasting immunity than that induced with oil adjuvant alone and improve both cellular and humoral immunity and resulted in earlier and more long lasting immunity, also it gave an early immunity when it used alone.

So, it is recommended to use FMD inactivated vaccine adjuvanted with oil and Clinoptilolite in companying of vaccination to control FMD.

References Références Referencias

1. *Abdel- Rahman, A. O.; Farag, M. A.; Samira El- Kilany; Eman, M. A.; Manal Abo El- Yazed and Zeidan, S. (2006):* Isolation and Identification of FMDV during an outbreak of 2006 in Egypt. Kafr El- Sheikh Vet. Med. J.; 4(1): 2006.
2. *Aikoh T, Tomokuni a, Matsukii T, Hyodoh F, Ueki H, Otsuki T, Ueki A (1998):* Activation-induced cell death in human peripheral blood lymphocytes after stimulation with silicate in vitro. Int J Oncol 12:1355-1359.
3. *Batista A, Quattrocchi V, Olivera V, Langellotti C, Pappalardo JS, DI Giacomo S, Mongini C, Portuondo DI, Zamorano P (2010):* Adjuvant effect of Cliptox on the protective immune response induced by an inactivated vaccine against foot and mouth disease virus in mice. Vaccine. 2010 Aug 31; 28(38):6361-6. doi: 0.1016/j.vaccine .2010. 06. 098. Epub 2010 Jul 14.
4. *Depa, P.M., Dimri, U., Sharma, M.C. and Tiwari, R. (2012):* Update on epidemiology and control of foot and mouth disease – A menace to international trade and global animal enterprise. Vet. World, 5(11): 694-70.
5. *Dar P, Kalaivanan R, Sied N, Mamo B, Kishore S, Suryanarayana VV (2013):* Montanide ISA 201 adjuvanted FMD vaccine induces improved immune responsesand protection in cattle. Vaccine 2013; 31:3327–32.
6. *David, 2013.* Evaluation of Calcium Phosphate Nanoparticles Mineralized with Proteins and Peptides for Use as Adjuvants in Protein and Nucleic Acid Vaccines. Doctor of Philosophy University of Washington 2013.
7. *Dong Li, Chunxue Zhou, Daliang She, Pinghua Li, Pu Sun, Xingwen Bai, Yingli Chen, Baoxia Xie, Zaixin Liu (2013):* The comparison of the efficacy of swine FMD vaccine emulsified with oil adjuvant of ISA 201 VG or ISA 206 VG, Journal of Biosciences and Medicines, 2013, 1, 22-25.
8. *Ehab El-Sayed Ibrahim, Wael Mossad Gamal, Amr Ismail Hassan, Safy El-Din Mahdy, Akram Zakria Hegazy and Magdy Mahmoud Abdel-Atty (2015):* Comparative study on the immunopotentiator effect of ISA 201, ISA 61, ISA 50, ISA 206 used in trivalent foot and mouth disease vaccine .Veterinary World, EISSN: 2231-0916 , P.(1189-1198).
9. *El-Din, W.M., Ibrahim, E.E., Daoud, H. and Ali, S.M. (2014)* Humeral and cellular immune response of Egyptian trivalent foot and mouth disease oil vaccine in cattle. Res. Opin. Anim. Vet. Sci., 4(4): 178-185.
10. *EL-Watany, H.; Shawky, M.M.; Roshdy, O.M. and El-Kelany, S. 1999.* Relationship between cellular and humoral immunity responses in animal vaccinated with FMD vaccine. Zagazig Vet. J., 27(1): 49-57.
11. *EL-Naggar, H. 2012.* Preparation of inactivated lyophilized NDV vaccine, M.V.Sc in Veterinary Science (Virology). Cairo University.
12. *European Parliament (1997):* Application for the Approval of Clinoptilolite Regulation (EC) No.258/97 of the European Parliament and of the Council of 27th January 1997.
13. *Fakhry, H.M., Rizk, S.A., Abu-Elnaga, H.I., Deghaidy, W., Talaat, A.A. and Hegazi, A.Z. (2012):* Field application of bivalent foot and mouth disease vaccine adjuvanted with Montanide ISA (25, 50, 206) and IMS (1113-3015) as an alternative to aluminum hydroxide gel. Egypt. J. Virol., 9(1): 123-136.
14. *Farag, M.A., Aggour, M.A. and Daoud, A.M. (2005):* ELISA as a rapid method for detecting the correlation between the field isolates of foot and mouth disease and the current used vaccine strain in Egypt. Vet. Med. J. Giza., 53(4): 949-955.
15. *Garces JM (1999):* Observations on Clinoptilolite applications. In: Treacz MMJ, Marcus BK, Misher ME,Higgins JB (eds) Proceedings of the 12th International Conference on Clinoptilolites. Materials Research Society, Warrendale, pp 551-566.
16. *Hamblin C, Barnett I T R and Crowther J R (1986):* Anew Enzyme-Linked Immuno Sorbent Assay (ELISA) for the detection of antibodies against FMD virus. П Application. Journal of immunological methods, 93: 123-129.
17. *Hiam M. Fakhry and Assem A A (2014):* study of zeolite as immune stimulant in foot and mouth disease trivalent oil adjuvant vaccine in cattle. 5th international conference of virology (December 9-12/2014), Egyptian J. virol. Vol. 11 (1) 60-69, (2014).
18. *Hiam F. Alhawary, Assem M. Abobaker, Sonia A. Rizk, Akram Z. Hegazi, Abobaker A. Agoor (2017):* Field application of trivalent foot and mouth disease vaccine adjuvant with Zeolite. Alhawary et al., J. of Virol. Sci., Vol. 2: 32-41, 2017.

19. *Huang, X., Li, Y., Fang, H. and Zheng, C. 2011:* Establishment of persistent infection with foot and mouth disease virus in BHK-21 cells. Virology Journal, 8: 169.
20. *Kre Imir Paveli , Mirko Had Ija , Ljiljana Bedrica , Jasminka Paveli , Ivan Điki Maakati , Marijeta Kralj , Maja Herak Bosnar , Sanja Kapitanovi, Marija Poljak-Blailmun Kri Anac , Ranko Stojkovi, Mislav Jurin, Boris Suboti And Miroslav Oli (2000):* Natural Clinoptilolite clinoptilolite: new adjuvant in anticancer therapy. Received: 17 April 2000/ Accepted: 15 October 2000 / Published online 1-2.
21. *Mercedes G. V.,Timothy D., Trevor C., Martin R. and R. Michael E. P. (1996):* Recognition of foot-and-mouth disease virus and its capsid protein VP1 by bovine peripheral T lymphocytes. Journal of General Virology (1996), 77, 727-735.
22. *Lombard, M.; Pastoret, P.P. and Moulin, A.M. (2007):* A brief history of vaccines and vaccination.Rev. Sci. Tech. 26 (1):29-48.
23. *Mansouri Nabiollah , Navid Rikhtegar, Homayon Ahmad Panahi, Farideh Atabi and Behrouz Karimi Shahraki 2013:* Porosity, characterization and structural properties of natural zeolite – clinoptilolite – as a sorbent. Environment Protection Engineering, Vol. 39 (139-152).
24. *Nader M. Sobhy, Sunil K. Mor, Mohammed E.M. Mohammed, Iman M. Bastawecy, Hiam M. Fakhry, and Sagar M. Goyal 2014:* Phylogenetic analysis of Egyptian foot and mouth disease virus endemic strains in 2013. 5th international conference of virology (December 9-12/2014), Egyptian J. virol. Vol. 11 (1) 49-59, (2014).
25. *OIE 2012:* Manual of diagnostic tests and vaccines for terrestrial animals. OIE, Paris, France: 2012. Foot and mouth disease. Chapter 2.1.5.
26. *OIE. (2013):* OIE/FAO Foot-and-Mouth Disease Reference Laboratory Network, Annual Report, 2013
27. *Orsel, K.; deJong, M.C.; Bouma, A.; Stegeman, J.A. and Dekker, A. (2007):* Foot and mouth disease virus transmission among vaccinated pigs after exposure to virus shedding pigs. Vaccine 2 21;25(34):6381-91
28. *Park JH. (2013):* Requirements for improved vaccines against foot-and-mouth disease epidemics. Clin Exp Vaccine Res 2013; 2:8–1.
29. *Pluimers, F.H. (2004):* Foot-and-Mouth disease control using vaccination: the Dutch experience in 2001.
30. *Reed, L.J. and Muench, H. (1938):* A simple method of estimating 50% end points. Am. J. Hyg., 27: 493-497.
31. *Ray Sahelian, M.D. (2016):* Zeolite supplement benefit and side effects, review, does it have benefits to the body. A review of Medline in 15 March 2016.
32. *Rhodes CJ (2010):* Properties and applications of Clinoptilolite. Sci Prog; 93(Pt3):223-84. Review PMID.
33. *Rodriguez, L.L. and Grubman, M.J. (2009):* Foot and mouth disease virus vaccines. Vaccine, 27 Suppl 4: D90-4.
34. *Sarkar A, Tamil Selvan RP, Kishore S, Ganesh K and, Bhanuprakash V 2017 :* Comparison of different inactivation methods on the stability of Indian vaccine strains of foot and mouth disease virus. Biologicals. 2017 Jul; 48:10-23.
35. *Scudiero, D.A., Shoemaker, R.H., Paull, K.D., Monks, A., Tierney, S., Nofziger, T.H., Currens, M.J., Seniff, D. and Boyd, M.R. 1988.* Evaluation of soluble tetrazolium/Formazan Assay for cell growth and drug sensitivity in culture using human and other tumer cell lines. Cancer Research Journal, 48: 4827-4833.
36. *Shawky M., Abd El-Aty M., Hiam. M. Fakry, Hind M. Daoud, Ehab El-Sayed I., Wael Mossad G., Sonia A. Rizk, Abu-Elnaga H., Mohamed A. A., Abd El-kreem A. and Farouk E. M. (2013) :* Isolation and Molecular Characterization of Foot and Mouth Disease SAT2 Virus during Outbreak 2012 in Egypt. J Vet Adv2013, 3(2): 60-68.
37. *Sonia A Rizk, Hiam M Fakhry and Abu-Elnaga H I (2010):* Comparative study of T cell proliferative response in cattle vaccinated with FMD vaccine using Cell titre-Aqueous one solution non radioactive assay (MTS). Zag. Vet. J. (ISSN. 1110-1458) Vol. 38, No. 4 pp. 188-195 (2010).
38. *Sonia, A.Rizk., Abo Bakr Agoor, Ekbal Farok, Hind daoud and Hiam M Fakhry. (2015):* Enhancing effects of Calcium phosphate nanoparticles adjuvant on the Immune response in cattle vaccinated with Foot and Mouth Disease trivalent vaccine. Egyptian J. virology vol.12 - 53- 66, (2015).
39. *Sharma, M.C., Pathak, M.N. Hung, M.N., Nhi D.L. and Vuc, N.V. (1984).*Report on the outbreak of foot and mouth disease in buffaloes in the southern part of Vietnam. Veterinary viral diseases: 302–305.
40. *Satya, P. (2009):* Vaccination against foot-and-mouth disease virus: Strategies and effectiveness. Expert Rev. Vaccines, 8(3): 347-365.
41. *Tsai CP, Pan CH, and Liu MY, Lin YL, Chen CM, Huang TS, (2000):* Molecular epidemio-logical studies on foot-and-mouth disease type O Taiwan viruses from the 1997epidemic. Vet Microbiol 2000; 74:207–1.
42. *Voller A, Bid Well D and Bartleha (1976):* Micro plate enzyme immuno assay for the immuno diagnosis of virus infection. In Manual of Clinical Immunology, Chapter 69, edited by N. Rose and H. Friedman, pp. 506- 512. American Society for Microbiology.
43. *Wisniewski J., Kobusiewiecz T., Baronowski C., and Jankowsko J.,(1972):* Determination of the level of immunity in cattle on the basis of neutralizing

antibodies after the use of a Frenkel type FMD vaccine. Medycyna Wet 28 (10): 586-588.

44. *Zahran, G.E.D. (1960):* Foot and mouth disease in southern region of URA. Bull. Off. Int. Epiz., 13: 390-393.0.

Table 1: Comparative delta optical density of the cell-mediated immune response of cattle, vaccinated with trivalent FMD vaccines using lymphocyte Proliferation (XTT) Assay

Time post vaccination	ΔOD in buffy coat in Vaccinated cattle			Control non vaccinated animal
	Group1 clinoptilolite	Group 2 (oil and clinoptilolite)	Group 3 (Oil)	
Pre vaccination	0.0488	0.0466	0.044	0.064
3rd day	0.5178	0.515	0.4736	0.065
1 week	0.8508	0.866	0.490	0.056
2 week	1.468	1.655	1.136	0.069
3 week	1.5572	1.660	0.856	0.067
4 week	1.257	1. 459	0.777	0.065
5 week	1.257	0.934	0.676	0.064
6 week	0.827	0.848	0.627	0.065
7 week	0.599	0.819	0.463	0.066

Table 2: Mean of serum antibody titers against type (O), (A) & SAT 2 in cattle vaccinated with trivalent FMD vaccines using SNT expressed log10

Weeks post vaccination	Cattle groups vaccinated with trivalent FMD vaccines									Non vaccinated Group
	FMD Clinoptilolite vaccine			FMD Clinoptilolite+ oil vaccine			FMD oil vaccine			
	Mean antibody titer against FMD virus strains									
	FMD (O)	FMD (A)	FMD (SAT2)	FMD (O)	FMD (A)	FMD (SAT2)	FMD (O)	FMD (A)	FMD (SAT2)	
Pre vacc	0.15	0	0.3	0.15	0.27	0.27	0.15	0.3	0.3	0.3
1	1.1	1.05	1.2	0.9	0.9	0.9	1.2	1.05	1.2	0.3
2	1.65	1.8	1.8	1.14	1.29	1.38	1.65	1.8	1.8	0.3
3	2.1	2.1	1.95	1.71	1.8	1.77	1.8	2.1	2.15	0.3
4	2.4	2.4	2.4	1.95	2.1	1.8	2.4	2.4	2.55	0.3
6	2.7	2.7	2.7	2.34	2.25	2.1	2.7	2.7	2.85	0.3
8	2.85	2.85	2.85	2.58	2.7	2.37	3.0	3.0	3.0	0.3
10	2.85	3.15	3.0	2.82	2.82	2.7	3.3	3.15	3.15	0.3
12	2.55	2.85	3.0	3.0	3.0	3.0	3.3	3.15	3.3	0.3
14	2.55	2.7	2.85	2.8	2.8	2.70	3.0	3.0	3.0	0.3
16	2.4	2.4	2.55	2.6	2.6	2.49	2.85	2.85	2.9	0.3
20	2.1	2.1	2.4	2.5	2.4	2.37	2.6	2.7	2.85	0.3
24	1.8	1.8	2.1	2.37	2.13	2.25	2.4	2.55	2.7	0.3
28	1.65	1.65	1.8	2.13	2.04	2.16	2.25	2.4	2.4	0.3
32	1. 5	1. 5	1.65	1.83	1.77	1.7	2.1	2.1	2.1	0.3
36	1.05	1.05	1.2	1.35	1.17	1.20	1.65	1.8	1.8	0.3
40	0.75	0.6	0.75	0.9	0.75	0.6	1.5	1.65	1.5	0.3

Table 3: Antibody titers of cattle vaccinated with inactivated trivalent FMD vaccine using ELISA against FMDV. Serotype (O, A and SAT2)

Time post vaccination	ELISA titers of vaccinated animal groups									Control group
	FMD Clinoptilolite vaccine			FMD Clinoptilolite+ oil vaccine			FMD oil vaccine			
	O	A	SAT2	O	A	SAT2	O	A	SAT 2	
0	0.18*	0.21	0.21	0.11	0.21	0.21	0.24	0.27	0.27	0.3
1 week	1.93	1.95	1.93	1.70	1.70	1.69	1.50	1.50	1.50	0.0
2 week	2.12	2.12	2.11	1.97	1.99	1.96	1.90	1.92	1.90	0.0
3 week	2.42	2.42	2.41	2.61	2.62	2.61	2.19	2.19	2.16	0.3
4 week	2.47	2.47	2.46	2.43	2.49	2.48	2.43	2.43	2.43	0.6
6 week	2.73	2.73	2.73	2.73	2.79	2.79	2.44	2.44	2.44	0.7
8 week	2.92	2.92	2.92	2.92	2.95	2.95	2.80	2.80	2.78	0.6
10 week	3.12	3.15	3.13	3.32	3.34	3.33	2.90	2.92	2.92	0.6
12 week	3.15	3.15	3.15	3.15	3.19	3.19	3.10	3.10	3.10	0.6
14 week	2.85	2.85	2.85	2.97	2.99	2.99	2.49	2.49	2.49	0.0
16 week	2.67	2.67	2.67	2.75	2.78	2.76	2.52	2.52	2.52	0.6
18 week	2.66	2.66	2.65	2.69	2.71	2.71	2.43	2.43	2.43	0.0
20 week	2.34	2.34	2.34	2.60	2.62	2.62	2.19	2.19	2.19	0.6
22 week	2.31	2.32	2.32	2.44	2.46	2.46	2.10	2.11	2.11	0.7
24 week	2.34	2.34	2.34	2.43	2.46	2.46	2.09	2.10	2.10	0.3
26 week	2.11	2.19	2.19	2.43	2.45	2.43	1.99	1.99	1.99	0.7
28 week	2.11	2.15	2.15	2.43	2.44	2.44	1.93	1.93	1.93	0.3
30 week	2.10	2.12	2.12	2.34	2.36	2.36	1.93	1.93	1.93	0.3
32 week	1.95	1.98	1.97	2.27	2.29	2.29	1.94	1.94	1.92	0.9
34 week	1.93	1.95	1.95	2.10	2.11	2.10	1.72	1.72	1.69	0.3
36 week	1.91	1.92	1.92	1.97	1.99	1.99	1.46	1.46	1.46	0.6
38 week	1.71	1.72	1.71	1.97	1.98	1.96	1.45	1.45	1.42	0.6
40 week	1.59	1.61	1.61	1.95	1.95	1.92	1.41	1.41	1.39	0.9

This page is intentionally left blank