



COMPARATIVE EVALUATION OF PREPARED INACTIVATED RIFT VALLEY FEVER VIRUS VACCINE WITH DIFFERENT ADJUVANTS

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ABSTRACT

Inactivated tissue culture adapted Rift Valley Fever (RVF) Virus vaccines were prepared using peanut oil; code liver oil, Montanide ISA 206 oil and aluminum hydroxide gel as adjuvants. The prepared vaccines were sterile and safe inducing no systemic or local clinical signs in lambs. Comparative evaluation of four experimentally prepared vaccines in sheep after a single dose indicated that the oily prepared vaccines greatly stimulated the humoral immune response as estimated by SNT and ELISA compared with aluminum hydroxide gel vaccine. Protective serum antibody titers against peanut oil and Montanide ISA 206 oil adjuvanted RVF virus vaccine started at 2nd week post vaccination (WPV) while code liver oil and Aluminum hydroxide gel vaccine started at 3rd WPV. These protective titers persisted till the 44th WPV for Montanide ISA 206 oil, 32th WPV for peanut oil and code liver oil and 24th WPV for Aluminum hydroxide gel vaccines then declined under the protective level. Both of SNT and ELISA indicated that among the used adjuvants, montanide ISA 206 was preferable as they could be used for manual preparation of the vaccines furthermore the superior immunological response followed by peanut oil then code liver oil and the aluminum hydroxide gel.

Key Words: RVF virus vaccines, Adjuvants, Sheep, SNT, ELISA

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1. INTRODUCTION

Rift Valley fever (RVF) virus, a Phlebovirus from the family Bunyaviridae, which is potentially transmitted by many different species of insect vectors that have a wide global distribution [1]. Periodic RVF outbreaks in livestock (goats, sheep, cattle, and camels) and acute febrile illness with hemorrhagic syndrome in humans have been reported widely throughout south and central Africa, from Kenya westward into Nigeria, Niger, Burkina Faso, Senegal, and Mauritania and northward into Egypt [2]. To limit spread of the disease, veterinary vaccines are the first line of defense against RVF virus infection. Extensive work has been carried out to produce safe and efficient vaccines against Rift Valley Fever. [3]. A trial for preparing a potent and safe

inactivated vaccine to be used for the spreading of the disease was attempted [4]. Two types of inactivated RVF vaccines were produced in Egypt; first produced by VACSERA company which is formalin inactivated and alum adjuvanted (Menya/Sheep/258), and the second produced by the Veterinary Serum and Vaccine Research Institute (VSVRI) which is binary ethylenimine (BEI) inactivated and alum hydroxide adjuvanted (ZH501 strain) [5] and [6].

The present trend in the development of new vaccines focuses on simplification of vaccination schemes either by increasing the components in the vaccines or by decreasing the number of vaccination doses needed to impart sufficient protection. One

of the possibilities how to achieve this goal is the use of new, more effective adjuvants. Adjuvant substances have been used for almost 80 years for increasing the effectiveness of various vaccines [7]. For a long time, oil adjuvants based on incomplete Freund's adjuvant played an important role in commercial veterinary vaccines. However, the mineral oil used caused various post-vaccination reaction of local or even general character in vaccinated individuals [8]. After replacing mineral oils with metabolisable natural oils (soya, sesame, olive, etc.) some local reactions were eliminated [9]. At the present time aluminium hydroxide is the substance most frequently used as adjuvant in veterinary medicine [10] although its potentiating effect fails to reach in general the level of oil adjuvants.

The present investigation dealt with the use of different oils (peanut oil, code liver oil and montanide ISA206 oil) as adjuvant with RVFV virus inactivated vaccine in order to induce high prolonged potent immunity in vaccinated sheep.

2. MATERIALS AND METHODS

2.1. Virus strain:

RVF virus Zagazig Human 501 (ZH501) strain was propagated in BHK-21 cell line of a titer $10^{7.5}$ TCID₅₀/ml was supplied by RVF Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. It was used for preparation of inactivated vaccine, SNT and ELISA.

2.2. Cell lines:

Baby hamster kidney (BHK-21) cell line established by [11]. They were used for propagation, titration of the virus and testing the safety of prepared inactivated virus suspension.

2.3. Animals:

2.3.1. Mice:

Forty five Albino Swiss mice, 3- 4 weeks old, were supplied by the Department of Pet Animal vaccine Research; Veterinary Serum and Vaccine Research Institute;

Abassia, Cairo. These mice were used in toxicity test to determine safe concentration of oil adjuvants.

2.3.2. Lambs:

Ten lambs of a range one month were apparently healthy and free from antibodies against RVFV as proved by using serum neutralization test. They kept under strict hygienic measures for studying the safety of the prepared vaccines.

2.3.3 Sheep and experimental design:

Twenty local breed sheep of nine to ten months old were used. These sheep were apparently healthy and free from antibodies against RVFV as proved by using serum neutralization test. The sheep were divided in five groups as shown in table (1):

Control group were non vaccinated and sheep was subcutaneously injected with physiological saline. All Sheep were housed in mosquito proof isolated stable and daily observed as well as body temperature was recorded.

Table (1): Groups of experimental sheep according to their type of vaccination:

Group	Prepared vaccine	sheep	dose	Route
I	Inactivated RVFV <i>peanut oil vaccine</i>	4	1 ml	S/C
II	Inactivated RVFV <i>code liver oil vaccine</i>	4	1 ml	S/C
III	Inactivated RVFV – <i>montanide ISA 206 vaccine</i>	4	1ml	S/C
IV	Inactivated RVFV- <i>aluminum hydroxide vaccine</i>	4	1ml	S/C
V	Control (<i>non-vaccinated</i>)	4	-	-

2.4. Serum samples:

All sera were collected from groups I, II, III, IV and V on the day of vaccination (zero day), then weekly till 48th week post vaccination where protective antibody level declined.

The sera were stored at -20°C and inactivated at 56°C for 30 minutes before being examined by the Serum Neutralization Test (SNT) and indirect enzyme-linked immunosorbent assay (ELISA).

2.5. Adjuvants:

2.5.1. *Peanut oil (Arachis oil)*: It was obtained from Sigma Company.Egypt. The chemical composition of peanut oil are: Iodine number 93.3 saponification number 205.5, fatty acids (Balmetic 8.3 Stearic 3.1, Arachidic 2.4 olic 56 and linolenic 26) according to [12]

2.5.2. *Cod liver oil (Squalene)*: It was obtained in pure form from a local market.

2.5.3. *Montanide ISA 206 VG*: It was obtained from SEPPIC, Cosmetics, Pharmacy Division, Paris, France. Bach NO 948400.

2.5.4. *Aluminum hydroxide gel (2%)* of low viscosity; stock No.2031200 was supplied under the name Rehydra gel by General Chemical Company; 235 Snyder Avenue, Berkeley Heights, New Jersey 07922.

2.6. *Toxicity test for adjuvants*: Three concentrations 10%, 20% and 30% were prepared for each of 3 adjuvants. Then 1ml from each concentration were inoculated intraperitoneally in 5 mice (0.2ml/mice) then observed for 10 days.

2.7. *Vaccine preparation*:

2.7.1. *Propagation of the RVF virus in BHK-21*: It was carried out according to [6].

2.7.2. *Titration of the virus in BHK-21*: it was done according to [6] and infectivity was calculated according to [13].

2.7.3. *Virus inactivation by 0.1% binary ethyleinimine*: Inactivation of RVF virus was done as described by [6].

2.7.4. *Preparation of inactivated RVF virus vaccines with different types of adjuvant*:

The aluminum hydroxide vaccine was prepared according to [6] while the peanut and Cod liver oil vaccines were prepared as water in oil (W/O) emulsion according to[14] and montanide ISA 206 oil vaccines water-in-oil-in-water (W/O/W) emulsion were prepared as [15].

2.8. *Quality control tests*:

Quality control tests were applied on the prepared inactivated gel and oil RVFVexperimental vaccine bathes including the freedom of foreign contaminants (aerobic and anaerobic bacteria; fungi and mycoplasma); safety and potency according to [16].

2.9. *Serum neutralization test (SNT)*: SNT was carried out using the micro-titer technique according to [17].

2.10. *Enzyme-linked immunosorbent assay (ELISA)*:

It was carried out according to [18] to determine antibodies against RVF virus using ELISA.

3. RESULTS

4.2. *Toxicity test*:

All types of oil adjuvant were safe in mice at concentration of 10%. Additionally montanide oil ISA 206 were non toxic at 20% and 40% oil concentration respectively table (2).

Table (2): Results of toxicity test of different oil adjuvant in mice

Types of oil	Number of inoculated mice	Mortality ratio in mice*		
		10%**	20%	40%
Peanut	15	0/5	1/5	1/5
Code liver	15	0/5	2/5	2/5
Montanide ISA 206	15	0/5	0/5	0/5

* Mortality ratio= dead/total inoculated mice per concentration, **Oil concentrations

4.2. Sterility test:

Inactivated vaccines were sterile as they were free from any bacterial and fungal contaminants.

4.3. Safety test of inactivated RVFV in lambs:

Inactivated vaccines were safe. It didn't produce any local or systemic reactions and also, no mortality in inoculated lambs

4.5. Humoral immune response in sheep vaccinated with different used oil inactivated RVF virus vaccines:

Serum neutralizing antibody titer against RVF virus were detectable at the protective level (1.7) by 2nd week post vaccination (WPV) in gP I and gP III, while the protective titer induced by gP II and gP IV were detectable by 3rd WPV.

Peak antibody titers were recorded by 20th WPV for gP I and gP III, 16th WPV for gP II and 12th WPV for gP IV. The protective titer extended till 44th WPV for gP III, 32th WPV for gP I and gP II and 24th WPV for gP IV indicating that Montanide oil ISA 206 induced the highest and long standing antibody titer followed by that of Peanut oil then code liver oil and lastly the aluminum hydroxide vaccine. The control group (gPV) showed absence of neutralizing antibodies along the period of the experiment (Fig 1) and (Table 3). RVF virus antibodies titer expressed with the optical density in sera of vaccinated sheep as observed by ELISA (Fig 2) and (Table 4) found parallel to preceding result in SNT.

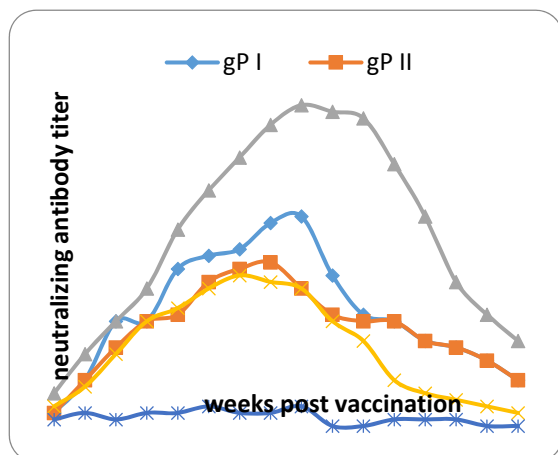


Fig. 1 Level of neutralizing antibody for RVFV induced by different inactivated RVFV vaccine adjuvanted with peanut oil(gPI) (◆), code liver oil(gPII) (■), montanide ISA 206(gPIII) (▲), and Aluminum hydroxide (gPIV) (×) in sera of sheep as assessed by SNT. (*) control unvaccinated (gPV)

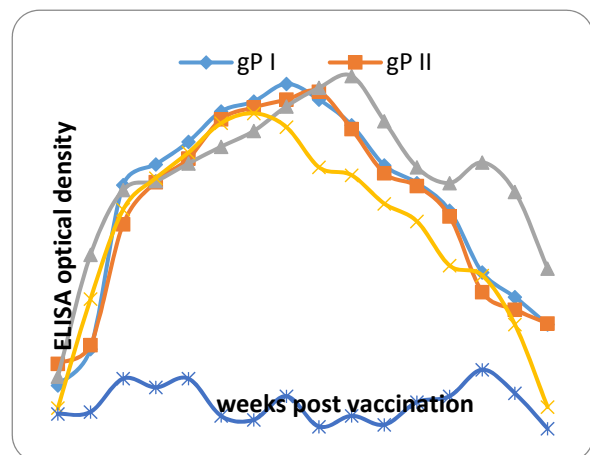


Fig. 2. Level of ELISA optical density for RVFV antibody induced by different inactivated RVFV vaccine adjuvanted with peanut oil(gPI) (◆), code liver oil(gPII) (■), montanide ISA 206(gPIII) (▲), and Aluminum hydroxide (gPIV) (×) in sera of sheep as assessed by ELISA. (*) control unvaccinated(gPV)

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Table (3): RVFV serum neutralizing antibody titers in vaccinated sheep with different RVFV adjuvant inactivated vaccines

	Mean RVFV serum neutralizing antibody titer*															
	Zero day	1 st **	2	3	4	8	12	16	20	24	28	32	36	40	44	48
gP I	0.3	0.8	1.7	1.7	2.5	2.7	2.8	3.2	3.3	2.4	1.8	1.7	1.4	1.3	1.1	0.8
gP II	0.3	0.8	1.3	1.7	1.8	2.3	2.5	2.6	2.2	1.8	1.7	1.7	1.4	1.3	1.1	0.8
gP III	0.6	1.2	1.7	2.2	3.1	3.7	4.2	4.7	5.0	4.9	4.8	4.1	3.3	2.3	1.8	1.4
gP IV	0.4	0.7	1.2	1.7	1.9	2.2	2.4	2.3	2.2	1.7	1.4	0.8	0.6	0.5	0.4	0.3
gP V	0.2	0.3	0.2	0.3	0.3	0.4	0.3	0.3	0.4	0.1	0.1	0.2	0.2	0.2	0.1	0.1

gPI: vaccinated with RVFV –peanut oil vaccine, gPII: vaccinated with RVFV –code liver oil vaccine, gPIII: vaccinated with RVFV –Montanide oil ISA206 vaccine, gPIV: vaccinated with RVFV– aluminum hydroxide gel vaccine, gPV: unvaccinated control, * neutralizing antibody titer expressed in log₁₀ TCID₅₀.** week post vaccination. The protective titer was 1.7log₁₀ according to Randall et al., [19].

Table (4): Mean RVFV -ELISA antibody titers in vaccinated sheep with different RVFV adjuvant inactivated vaccines

	Mean ELISA optical density for RVFV antibody *															
	Zero day	1 st **	2	3	4	8	12	16	20	24	28	32	36	40	44	48
gP I	0.048	0.085	0.252	0.273	0.296	0.327	0.337	0.355	0.339	0.313	0.272	0.254	0.226	0.163	0.138	0.110
gP II	0.07	0.089	0.212	0.255	0.279	0.319	0.331	0.339	0.347	0.309	0.264	0.251	0.220	0.143	0.125	0.111
gP III	0.057	0.181	0.247	0.256	0.274	0.291	0.307	0.332	0.351	0.363	0.317	0.270	0.254	0.275	0.245	0.167
gP IV	0.025	0.136	0.227	0.259	0.285	0.315	0.325	0.311	0.270	0.262	0.233	0.215	0.170	0.160	0.110	0.026
gP V	0.019	0.021	0.055	0.046	0.055	0.017	0.013	0.037	0.006	0.017	0.008	0.031	0.037	0.064	0.04	0.004

gPI: vaccinated with RVFV –peanut oil vaccine, gPII: vaccinated with RVFV –code liver oil vaccine, gPIII: vaccinated with RVFV –Montanide oil ISA206 vaccine, gPIV: vaccinated with RVFV– aluminum hydroxide gel vaccine, gPV: unvaccinated control, * Optical density values of ELISA with positive results above 0.253 (Cut off value). ** Week post vaccination

4. DISCUSSION

Often vaccinologists search and aim to improve vaccines to overcome the obstacles which may face the older vaccines such as the unsafely or the low induced immunity. The use of adjuvant plays the greater role in this field. The present work is a trial to improve the locally produced inactivated RVFV vaccine through the use of three different adjuvants other than the used aluminum hydroxide gel.

The experimental results revealed that all prepared vaccine formulae were free from foreign contaminants (aerobic and anaerobic bacteria; fungi and mycoplasma) and safe in vaccinated animals where such animals remained healthy all over the experimental period without local reaction at the site of inoculation. These observations agree with the recommendations of [16]. Also [20] showed that an adjuvant should stimulate high antibody titers, but in the process it should have low toxicity and not induce harmful side effects after injection. For the same purpose, [14] replaced the mineral oil by animal and vegetable oils in the inactivated ND vaccine recording high levels of antibodies. Also [21] found that W/O/W did not cause local reaction at the site of inoculation.

Results of SNT (Table 3) and (Fig 1) and ELISA (Table 4) and (Fig 2) were coming in a parallel manner confirming each other indicating that Montanide oil ISA 206 induced the highest and long standing protective antibody titer followed by that of Peanut oil then code liver oil and lastly the aluminum hydroxide gel vaccine. The protective level of neutralizing antibodies was evaluated according to [19], who suggested that the protective titre against RVF virus was $1.7 \log_{10}$.

Higher SNT titer and ELISA optical density reported that oil based RVF virus vaccines compared with that of aluminum hydroxide gel containing vaccines could be explained by the relatively rapid release of the antigen from the gel material in

comparing with that in oil adjuvant trapped by oil environment [22].

Peanut oil based formulations are potent immunological adjuvants induced minimal adverse side-effects [23]. increased the synthesis of anti-RVF virus antibodies were came in agreement with [24] who suggested that peanut oil stimulated the Th2 immune response and down regulated Th1 response and thus significantly increase the synthesis of antibodies in the primary response, but it did not favor cellular response.

cod liver oil contains a branched, unsaturated terpenoids known as squalene that very effective in potentiating the efficacy of inactivated rabies vaccines or of porcine parvovirus vaccine [25] and our results recommended this metabolisable adjuvant for the purpose of potentiating the effect of RVF virus inactivated veterinary vaccines without the risk of development of post vaccination complications in animals

On the other hand Montanide oil ISA 206 induced the highest and the probably longest immunity as stated by [26] and [27] as this adjuvant improves and enhance both cell mediated and humoral immune response [28].

In conclusion, oil inactivated formulae of RVF virus vaccine induced higher levels of immunity than that induced by the aluminum hydroxide vaccine but on specification montanide ISA were adjuvants of choice not only for their results but also their ready to use which allow the manufacture of different type of emulsion: water in oil, oil in water or water in oil in water they can be based on mineral oil, on mineral oil or a mix of them. These adjuvants are patent contain its own surfactant which enable an easy manufacturing of vaccines by mixing the aqueous medium into the montanide oil at room temperature.

5. REFERENCES

1. Gubler, D. J. 2002. The global emergence/resurgence of arboviral

- diseases as public health problems. *Arch. Med. Res.* 33,330–342.
2. Diallo, M., Nabeth, P., Ba, K., Sall, A., Ba, Y., Mondo, M., Girault, L., Abdalahi, M. and Mathiot, C. 2005. Mosquito vectors of the 1998–1999 outbreak of Rift Valley Fever and other arboviruses (Bagaza, anar, Wesselsbron and West Nile) in Mauritania and Senegal. *Med. Vet. Entomol.* 19, 119–126.
 3. Kamal, S.K. 2011. Observations on rift valley fever virus and vaccines in Egypt. *Viol. J.* 8: 532-540.
 4. Abd El Samea, M. M.; Elian, K. and Gihan, K. M. 1994. The effect of Rift Valley Fever and sheep pox vaccines on the immune response of sheep. *J. Egypt. Vet. Med. Ass.* 2:129-136.
 5. El-Nimr, M.M. 1980. Studies on the inactivated vaccine against RVF. Ph. D. Thesis (Microbiology). Fac. Vet. Med Egypt: Assiut University.
 6. Eman. M. S. S. 1995. Studies on RVF vaccine inactivated with Binary. Ph. D. Thesis (Microbiology). Fac. Vet. Med Egypt: Cairo University.
 7. Ramon G. 1925: Sur l'augmentation anormale de l'antitoxine chez les chevaux producteurs de serum antidiphthérique. *Bull Soc Centr Méd Vét.* 101:227–234.
 8. Vanselow BA. 1987. The application of adjuvants to veterinary medicine. *Vet Bull.* 57:881–96.
 9. Reynolds JA, Harrington DG, Crabbs CL, Peters CJ, Di Luzio NM. 1980. Adjuvant activity of a novel metabolisable lipid emulsion with inactivated viral vaccines. *Infect Immun;* 28: 937–943.
 10. Clements CJ, Griffiths E. 2002. The global impact of vaccines containing aluminium adjuvants. *Vaccine;* 20: 24–33.
 11. Mackpherson, L.A. and Stocker, M.G.P. 1962. Polyoma transformation of hamster cell colonies; An investigation of genetic factors affecting cell competence. *Virology*, 16: 147.
 12. Walker M. E. and Keisling T. C. 1978. Response of Five Peanut Cultivars to Gypsum Fertilization on Soils Varying in Calcium Content. *Peanut Science.* 5: 57-60.
 13. Reed, L.J. and Muench, H. 1938. A simple method for estimating 50 percent end point. *Am. J. Hyg.*, 27: 493 - 497.
 14. Stone, H.D.; Brugh, S.R.; Hopkins, H.W.; Yoder, C. and Bread, C.W. 1978. Preparation of oil emulsion vaccine with avian viral or mycoplasmal antigen. *Avian Dis.*, 22: 666-674.
 15. Barnett, P., Pullen, L. Williams, L. and Doel, T.R. 1996. Assessment of Montanide ISA 25 and 206, two commercially available oil adjuvant. *Vaccine.* 14: 1187- 1198.
 16. USA-CER 1987. Code of Federal Regulation; United States of America.
 17. Ferreira, M.E. 1976. Prubade micro-neutralization proestuose de anticurpos de la fiebre aftose. Blth. Centropan Americano Fieber Aftosa, 21 and 22: 17-24.
 18. Voller, A., Bidwell, D.E. and Annbarlett, M. 1976. Enzyme immuno assays in diagnostic medicine, theory and practice. *Bull. World. Health, organ*, 63: 55-65.
 19. Randall, R.; Binn, L. N. and Harison, V. R. 1964. Immunization against Rift Valley Fever virus. Studies on the immunogenicity of lyophilized formalin inactivated vaccine. *J. Imm.*, 93 (2): 293-299.
 20. Gupta, R.K.E.; Relyveld, E.H.; Lindblad, E.B.; Bizzini, B.; Ben-Efrain, S. and Gupta, C.K. 1993. Adjuvant-a balance between toxicity and advuvanicity. *Vaccine*, 11: 293-306.
 21. Amani, A.Saleh 2006. Comparative studies on the immunogenicity of inactivated bovine ephemeral fever vaccine using oil and aluminum hydroxide gel as adjuvants. *Minufiya Vet.Med.J.* 4 (2): 389-394.
 22. Jansen, T.; Hofmans, M.P.; Theelen, M.J. and Schijns, V.E. 2005. Structure-activity relations of water-in-oil vaccine formulations and induced antigen-specific antibody responses. *Vaccine* 23:1053-1060.
 23. Eghafona N.O. 1996. Immune response following cocktails of inactivated measles vaccine and *Arachis hypogea* L. (groundnut) or *Cocos nucifera* L. oils adjuvant. *Vaccine;* 14:1703-1708.
 24. Freitas, E., Marinho, A. C., Albuquerque, D., Teles L., Sindeaux, M., Salles, M. T.,

- Sousa, D. C., Lima, M. M., Silva, M. G., Fernandes, D. 2013. Adjuvant activity of peanut, cottonseed and rice oils on cellular and humoral response. *VacciMonitor*. 22:4-9.
25. Süli J, Benčíšek Z, Eliáš D, Švrček Š, Ondřejková A, Ondrejka R, Bajová V. 2004. Experimental squalene adjuvant. I. Preparation and testing of its effectiveness. *Vaccine*. 22:3464–9.
26. Fatthia, A.M. 2003. Vaccination of goats with FMD vaccines. MVSc Thesis (Infectious disease), University of Alexandria.
27. Sonia, A.M. 2007. Studies on preparation improved FMD Virus oil adjuvant vaccine. MVSc Thesis (Virology), University of Cairo.
28. Aucouturier, J., Ascarateil, S., Dupuis, L., 2006. The use of oil adjuvants in therapeutic vaccine. *Vaccine* 24 :44–45



التقييم المقارن للقاح فيروس حمى الوادي المتصدع المثبط المحضر مع ممتزجات مختلفة

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الملخص العربي

تم تحضير لقاحات مثبتة لفيروس حمى الوادي المتصدع المأقلم على خلايا الزرع النسيجي وممتزجه بزيت الفستق او زيت كبد الحوت او زيت المونتانيدي 206 او جل هيدروكسيد الالمونيوم. اثبتت النتائج ان اللقاحات المحضرة كانت نقية خالية من مسببات المرضية وامنة. بمقارنة نتائج تقييم الاربعة لقاحات المحضرة فى الاغنام باستخدام جرعة واحدة من كل منها تبين ان اللقاحات المحضرة باستخدام الممتزجات الزيتية أكثر تحفيزا للاستجابة المناعية الخلطية مقارنة بلقاح جل هيدروكسيد الالمونيوم وذلك عند قياسها باختبارى المصل المتعادل والأنزيم الممدص المرتبط المناعي. بدأت عيارية الاجسام المضادة الواقية لكل من لقاح حمى الوادي المتصدع الممتزج بزيت الفستق وزيت المونتانيدي 206 عند الاسبوع الثاني من التحصين بينما كانت عند الاسبوع الثالث من التحصين لكل من لقاح حمى الوادي المتصدع الممتزج زيت كبد الحوت وجل هيدروكسيد الالمونيوم. استمرت العيارية الواقية حتى الاسبوع 44 بعد التحصين للقاح الممتزج بزيت المونتانيدي 206 و الاسبوع 32 بعد التحصين للقاح الممتزج بزيت الفستق وزيت كبد الحوت والاسبوع 24 بعد الحقن للقاح الممتزج بجل هيدروكسيد الالمونيوم ثم انحدرت العيارية الواقية للأجسام المضادة بعد ذلك. اشار كلا من اختباري المصل المتعادل والأنزيم الممدص المرتبط المناعي ان زيت المونتانيدي 206، هو الافضل فى تحضير اللقاحات الزيتية مقارنة بالأنواع الاخرى حيث يمكن استخدامهما فى خلط اللقاح يدويا بالإضافة الى المستوى المناعي المتميز ويليه كلا من زيت الفستق ثم زيت كبد الحوت و جل هيدروكسيد الالمونيوم.

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