EVALUATION OF INACTIVATED RIFT VALLEY FEVER VACCINE WITH PARAFFIN OIL ADJUVANT

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A B S T R A C T

Rift Valley Fever (RVF) caused by an arbo-virus belonged to genus Phlebovirus, family Bunyaviridae, is an acute, febrile disease of ruminants. In Egypt, immunization of susceptible animals occurs using a locally prepared inactivated vaccine with aluminum hydroxide gel adjuvant. Adjuvants play an important role in vaccine formulation, so, selection of the proper adjuvant can elaborate high and long standing immunity. The aim was to develop a new RVF vaccine with paraffin oil adjuvant in water in oil in water (W/O/W) formula using Tween80 and Arlacel A as surfactants. Evaluation of the immune response to the prepared RVF vaccine in sheep was done in comparison with the local RVF vaccine with using SNT and ELISA. Results gave the priority to the prepared inactivated RVF vaccine with Paraffin oil adjuvant that induced high immunological enhancement without toxicity and with longer duration of immunity that extended for 9 months

Key Words: RVF vaccine, paraffin oil, SNT

(BVMJ 24(2): 157-164, 2013)

1. INTRODUCTION

Rift Valley Fever (RVF) disease is an acute, febrile disease characterized by hepatitis and high mortality in lambs and calves, abortion in adult sheep and cattle, and by influenza-like disease or hemorrhagic fever in human [1].

The disease is caused by a mosquito-borne RVF virus belonged to genus Phlebovirus in the *Bunyaviridae* family [2]. It is an enveloped virus, has a single-stranded tripartite RNA genome composed of large (L) segment that codes for the polymerase, medium (M) segment that codes for glycoproteins (Gn and Gc) and non-structural proteins (NSm14 and small

(S) Segment that code for nucleocapsid. Glycoproteins are the targets for neutralizing antibodies and influence virus cell attachment [3]. Antigenic properties of the glycoproteins and nucleoprotein appeared to be stable in natural RVF virus isolates [4]. The first outbreak of RVF in Egypt was recorded in animals and human at Sharqiya Governorate in 1977 [5], [6], then it re-emerged in the years 1993, 1994, 2003 and 2011 [7], [8], [9], [10]. Control of vectors and vaccination of susceptible animals were the most effective methods of RVF control, because countries have had an outbreak in the past, would have a very high likelihood of future outbreaks [11]. There are two types of vaccines that are generally used, the modified live vaccine and an inactivated cell culture vaccine. In Egypt, inactivated RVF vaccine with aluminum hydroxide gel adjuvant has been used for vaccination of susceptible animals [12], [13].

Our study aimed to improve the quality of economic oils such as paraffin oil as an

adjuvant in a trial to produce an emulsion vaccine of high quality, prepared in the formula of water in oil in water (W/O/W) using a surfactant blend of Arlacel A (a nonionic surfactant with low HLB value produces an easy-flowing injectable emulsion), and Tween 80 (A nonionic surfactant with high HLB value).

2. MATERIALS AND METHODS

2.1. *Virus:*
RVF virus ZH501 strain propagated in Baby Hamster Kidney (BHK-21) cells with a final titre107.5 TCID50 / ml, was obtained from RVF vaccine research department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. It was used in vaccine preparation and as a reference infective RVF virus for serum neutralization test (SNT). It was kept at -70˚C.

2.2. *Baby Hamster Kidney (BHK21) Cell Culture:*
It was used for propagation and titration of RVF virus and also used for SNT.

2.3. *Vaccines:*

2.3.1. Inactivated RVF vaccine with aluminum hydroxide gel adjuvant
It is a locally prepared RVF vaccine [12] supplied by RVF vaccine research department, Veterinary Serum & Vaccine Research Institute, Abbassia.

2.3.2. Prepared Inactivated RFV vaccine adjuvant with paraffin oil.

a) Inactivation of the virus
RVF virus ZH501 propagated in BHK-21 cells, harvested and titrated in BHK-21 cells and the seed virus with a final titre107.5 TCID50 / ml, was inactivated using 0.0001 M of Binary ethylinimine (BEI) [12], Then over action of BEI was stopped by sterile 20% sodium thiosulphate solution.

b) Inactivation of the virus
Samples from the inactivated virus were checked for residual infectivity; by inoculation on BHK-21 cell and in baby mice by intracerebral route. Absence of cytopathic effect on cell culture and absence of mortality in baby mice indicated optimum inactivation process.

c) Testing safety of the paraffin oil, Arlacel A and Tween80
Safety of paraffin oil adjuvant and Arlacel A and Tween80 surfactants were tested by intra-peritoneal inoculation (I/P) into groups of adult mice in different percentages with saline. Mice were kept under observation for 10 days.

d) Preparation of the oil emulsion RFV vaccine
Oil emulsion RFV vaccine was prepared in a W/O/W formula using paraffin oil with a surfactant blend of 60% tween80 and 40% Arlacel A according to HLB (Hydrophyl Lipophyl Balance) rules produces an easy-flowing injectable emulsion [14]. Merthiolate was prepared as 10% solution and used at a final concentration 1:10000 in the prepared vaccine as bactericidal agent.

e) Testing quality of the oil emulsion RFV vaccine
The vaccine emulsion was tested with the organoleptic method, it showed homogenicity of the emulsion and typical color of the product after it was stored at room temperature for 24 hours and at 4 – 8 °C for two weeks. Then testing sterility, safety and potency of the vaccine was performed [15].

2.4. *Sheep and experimental design:*
Fourteen susceptible balady sheep (4 – 6 months old), healthy, clinically normal, and free from antibodies for RVF virus were used for potency evaluation of the inactivated RVF vaccines as follow:
Group 1: contain 6 animals each was vaccinated by subcutaneous inoculation with 1ml of inactivated RVF oil adjuvant vaccine.

Group 2: contain 6 animals each was vaccinated by subcutaneous inoculation with 1ml of inactivated RVF vaccine adjuvanted with Aluminum hydroxide gel.
Group 3: contain 2 animals kept as non-vaccinated control.
All animals were kept under close observation during the whole time of experiment and subjected for serum samples collection.

2.5. *Serum samples:*
Serum samples were collected from sheep under study weekly for 4 weeks, then monthly till 12 months. The collected sera were stored at -20°C and inactivated at 56°C for 30 minutes before being used in the test.

2.6. *Serum neutralization test (SNT):*
Detection of the specific neutralizing antibodies against RVF virus in the serum samples of vaccinated sheep were done according to method of constant serum-virus dilution procedure [16], and the neutralizing index of serum was calculated [17]

2.6. *Indirect Enzyme Linked Immunosorbent Assay (indirect ELISA)*
Reagents of ELISA were prepared [18], with the use of rabbit anti-sheep immune-globulins labeled with horse-reddish peroxidase (HRPO) enzyme. Procedures were done [19] and positive serum samples have optical density equal to or greater than the calculated cut off value [20].

3. RESULTS

3.1. Potency of the prepared vaccine in mice:
Potency of the two RFV vaccines were calculated in mice as 0.0002 ED_{50}/ml and 0.0013 ED_{50}/ml for the prepared inactivated RVF vaccine with paraffin oil adjuvant and the local inactivated RVF vaccine with aluminum hydroxide gel.

3.2. *Clinical examination of vaccinated sheep:*
The result shown that there were no clinical signs and no elevation of body temperature for 7 days post vaccination in all vaccinated groups in comparison to the control group.

3.3. *Evaluation of humeral immune response in vaccinated sheep:*

a) Using SNT
Mean neutralizing index in sera from vaccinated sheep using the prepared RVF vaccine with Paraffin oil adjuvant reached a protective level (1.7) at the 2nd week post vaccination, increased gradually till to reach its peak (2.9) at 3rd month post vaccination and maintained with the protective level (1.5) at the 9th month post vaccination and then decline to a non-protective level (below 1.5).
In comparison, mean neutralizing index in sera from vaccinated sheep using the local RVF vaccine with aluminum hydroxide gel adjuvant reached a protective level (1.5) at the 2nd week post vaccination, increased gradually till to reach its peak (2.6) at 3rd month post vaccination and maintained with the protective level (1.5) at the 7th month post vaccination and then decline to a non- protective level (below 1.5). These results were compared with those of the non-vaccinated control sheep as shown in table (1) and figure (1).

b) Using ELISA
Mean optical density in sera from vaccinated sheep using the prepared RVF vaccine with Paraffin oil adjuvant started to appear in positive level at 2nd week post vaccination, reached to the peak at the 3rd month post vaccination and continued at positive level till 9th month post vaccination then began to decline under the positive values.
In comparison, mean optical density in sera from vaccinated sheep using the local RVF vaccine with aluminum hydroxide gel started to appear in positive level at 2nd week post vaccination, reached to the peak at the 3rd month post vaccination and continued at positive level till 6th month post vaccination then began to decline

under the positive values. These results were compared with those of the non-vaccinated control sheep as shown in table (2) and figure (2).

Table 1. Neutralizing Index in sera from vaccinated sheep

Animal group	Mean Neutralizing Index															
	* BV	Weeks post vaccination				Months post vaccination										
		1st	2nd	3rd	4th	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
G1	0.34	0.96	1.75	1.9	2.2	2.6	2.9	2.7	2.5	2.3	2.1	1.8	1.5	1.3	1.1	0.9
G2	0.43	0.9	1.6	1.8	2.0	2.6	2.4	1.9	1.8	1.7	1.7	1.1	0.9	0.7	0.6	0.6
G3	0.7	0.7	0.65	0.7	0.7	0.7	0.65	0.65	0.65	0.7	0.7	0.65	0.65	0.7	0.7	0.65

*G1: Group of sheep vaccinated with inactivated RVF oil adjuvant vaccine. G2: Group of sheep vaccinated with inactivated RVF aluminum hydroxide gel adjuvant vaccine.G3: Group of sheep none vaccinated kept as control.**BV: Before vaccination.Protective neutralizing index is (1.5).

Table 2. ELISA optical density in sera from vaccinated sheep

group	Mean ELISA Optical Density															
	* BV	Weeks post vaccination				Months post vaccination										
		1st	2nd	3rd	4th	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
G1	0.026	0.165	0.292	0.319	0.330	0.344	0.366	0.357	0.346	0.339	0.324	0.316	0.297	0.278	0.231	0.178
G2	0.032	0.162	0.290	0.319	0.338	0.358	0.349	0.330	0.319	0.292	0.273	0.257	0.242	0.231	0.221	0.215
G3	0.039	0.034	0.039	0.042	0.037	0.036	0.041	0.038	0.041	0.038	0.039	0.042	0.063	0.037	0.041	0.039

*G1: Group of sheep vaccinated with inactivated RVF oil adjuvant vaccine.G2: Group of sheep vaccinated with inactivated RVF aluminum hydroxide gel adjuvant vaccine.G3: Group of sheep none vaccinated kept as control.

**BV: Before vaccination. Cut off value of ELISA optical density is (0.288).

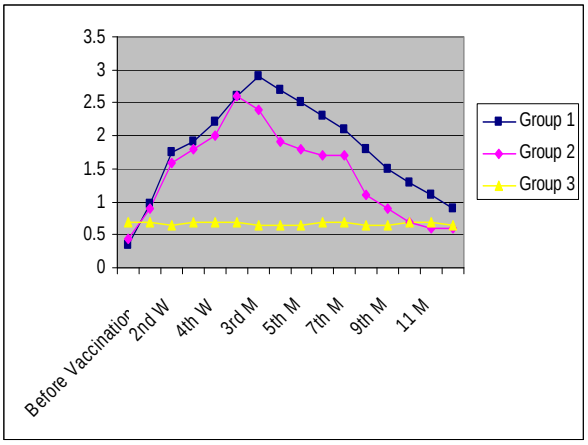

Fig (1): Duration of immunity in vaccinated sheep measured with SNT

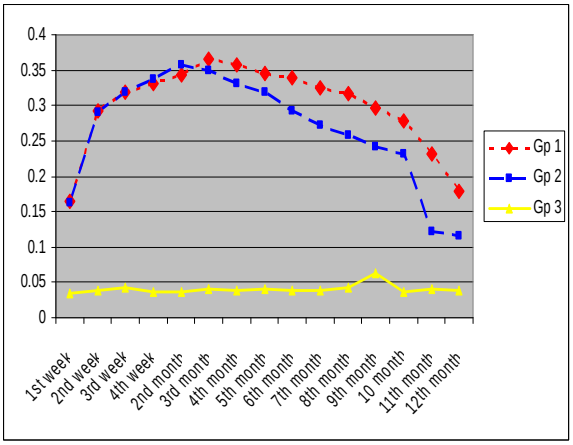

Fig (2): Duration of immunity in vaccinated sheep measured with ELISA

4. DISCUSSION

Adjuvants play an important role in vaccine formulation, that can elaborate high and long standing immunity. Vaccine with aqueous adjuvant may have short shelf-life validity and need for one or more boostering doses to maintain an adequate level of specific antibodies, So, several trials were done to switch to oil formulations as pea nut oil, nigella sativa oil and paraffin oil [21], [22]. Water in oil in water emulsions can combine the advantage of oil in water formulation by inducing an earlier response as well as the advantage of water in oil formulations by inducing a long term immune response. Moreover, a low shear mixing equipment is required to get a stable formulation or even a simple homogenizer is recommended and the emulsion is done in a one step process [23].

Calculation of potency for RVF vaccines in mice were 0.0002 ED_{50}/ml and 0.0013 ED_{50}/ml, for the prepared inactivated RVF vaccine with paraffin oil adjuvant and the local inactivated RVF vaccine with aluminum hydroxide gel adjuvant, respectively. These results agreed with those reported that the protective ED_{50}/ml for RVF vaccine should be less than 0.02/ml, and thus the lower ED_{50} values, the more potent are the vaccine [24].

Sheep were inoculated with both types of vaccine at a dose of 1ml by subcutaneous route did not show any post-vaccination clinical signs or elevation in temperature. These result agreed with those who used inactivated RVF vaccines without any post-vaccinal reaction in inoculated animals [12], [25], [26].

Evaluation of humeral immune response in vaccinated sheep studied by SNT showed that mean neutralizing index (NI) in sera from vaccinated sheep started to rise from 1st week post vaccination and increased to the protective level at 2nd week post vaccination with both prepared inactivated RVF vaccine with paraffin oil adjuvant and the local inactivated RVF vaccines with aluminum hydroxide gel adjuvant as shown in table (1) and figure (1). These results agree with those who recorded that the protective NI level obtained by the inactivated vaccines was 2 weeks post vaccination [12], [25], [27] and also agree with those who found that the neutralizing antibodies in sheep and goats vaccinated with formalin inactivated RVF vaccine could be detected 7 days post vaccination [28].

Mean NI in sera from vaccinated sheep increased gradually till reached the peak at the 3rd month and 2nd month post vaccination, and then the duration of protective level extended to the 9th month and 7th month post vaccination with the prepared inactivated RVF vaccine with paraffin oil adjuvant and the local inactivated RVF vaccines with aluminum hydroxide gel adjuvant, respectively, then decline to a non-protective level (below 1.5). These results gave the priority to the adjuvanted vaccine with Paraffin oil over the vaccine adjuvanted with aluminum hydroxide gel. These results were compared with those of the non-vaccinated control sheep as shown in table (2) and figure (2). These results come in agreement with those who recorded that double oil emulsion Foot and Mouth Disease vaccine, elicited superior immune response than the aluminum hydroxide gel vaccine and the development of immune response was quicker [29], [30].

Evaluation of humeral immune response in sera from vaccinated sheep using ELISA showed that the mean optical density in sera from vaccinated sheep started to appear in positive level at 2nd week post vaccination for both types of vaccines then mean optical density reached to the peak at the 3rd month and 2nd month post vaccination for vaccinated sheep with the prepared inactivated RVF vaccine with Paraffin oil adjuvant and the local inactivated RVF vaccine with aluminum hydroxide gel adjuvant, respectively. The duration of

positive level in sera from vaccinated sheep extended to the 9th month and 6th month post vaccination with the prepared inactivated RVF vaccine with paraffin oil adjuvant and the local inactivated RVF vaccines with aluminum hydroxide gel adjuvant, respectively, then it decline to a negative level. These results were compared to that of non- vaccinated control sheep as shown in table (2) and figure (2). The result of ELISA was correlated with that obtained by SNT. These results come in agreement with those who used ELISA for detection of IgG instead of SNT. They demonstrated that ELISA is a guide test, which is safe and useful for monitoring of immune response after vaccination [31], [32], [33].

From the previous results we can conclude that the newly prepared inactivated RVF vaccine with paraffin oil adjuvant induced immunological enhancement without toxicity and gave higher titer of antibody that remained for a much longer duration in the period of immunity compared with that of the local inactivated RVF vaccine with aluminum hydroxide gel adjuvant. Also it has an important advantage when used in large scale as it is easily prepared with low cost.

5. REFERENCES

1. Radostits, O.M., Gay, C.C., Hinchcliff, K. W. and Constable, P.D. 2008. Part 2 Special Medicine. Chapter, 21, Diseases associated with viruses"Rift Valley Fever", In Veterinary medicine, A Textbook of The Diseases of Cattle, Horses, Sheep, Pigs and Goats. 10th Edn., W.B. Sounders, London, New York, Oxford, pp.1205 – 1207.
2. Vialat, P., Billecocq, A., Kohl, A. and Bouloy, M. 2000. The S segment of rift valley fever phlebovirus (Bunyaviridae) carries determinants for attenuation and virulence in mice. J Virol, 74: 1538-43.
3. Yadani, F.Z., Kohl, A., Préhaud, C., Billecocq, A. and Bouloy M. 1999. The carboxy-terminal acidic domain of Rift Valley Fever virus NSs protein is essential for the formation of filamentous structures but not for the nuclear localization of the protein. J Virol, 73: 5018-5025.
4. Ihara, T., Akashi, H. and Bishop, D.H.L 1984. Novel Coding Strategy (Ambisense Genomic Rna) Revealed by Sequence Analyses of Punta Toro Phlebovirus-S Rna. Virology, 136: 293-306.
5. Imam, Z. E. and Darwish, M. A. 1977. A preliminary report on an epidemic of Rift Valley Fever (RVF) in Egypt. J Egypt Public Health Assoc.; 52: 417–418.
6. Imam, Z. E. Imam, El-Karamany, W. R. and Darwish, M. A. 1979. An epidemic of Rift Valley fever in Egypt. 2. Isolation of the virus from animals, Bulletin of the World Health Organization, 57: 441-443.
7. Arthur, R.R., El Sharkawy, M.S., Cope, S.E., Botros, B.A., Oun, S., Morrill, J.C., Shope, R.E., Hibbs, R.G., Darwish, M.A. and Imam, I.Z.E. 1993. Recurrence of Rift valley Fever in Egypt. Lancet 342:1149–1150.
8. Abd-El-Rahim, I.H., Abd-El-Hakim, U. and Hussein, M. 1999. An epizootic of Rift Valley fever in Egypt in 1997. Rev Sci Tech, 18:741-748.
9. WHO 2003. World Health Organization, Disease outbreak reported: Rift Valley fever in Egypt. Weekly Epidemiological Record 36:5.
10. Hanafi, H.A., Fryauff, D.J., Saad, M.D., Soliman, A.K., Mohareb, E.W., Medhat, I., Zayed, A.B, Szumlas, D.E. and Earhart, K.C. 2011. Virus isolations and high population density implicate, culex antennatus, (Becker) (Diptera: Culicidae) as a vector of Rift Valley fever virus during an outbreak in the Nile Delta of Egypt. Acta Tropica, 119:119-124.
11. Gerdes, G.H. 2004. Rift Valley fever. Revue Scientifique Technique Office International Des Epizooties. 23:613-623.
12. Eman. M. S. S. 1995. Studies on RVF vaccine inactivated with Binary. Ph. D. Sc. thesis Microbiology, Fac. of Vet. Med. Cairo -Univ.
13. Samia, A. Kamal 2011. Observations on rift valley fever virus and vaccines in Egypt. Virology Journal, 8:532 – 540.
14. Tengerdy, R.P. and Lacetera, N.G. 1991.Vitamine E adjuvant formulations in mice. Vaccine, 9:204-206.
15. OIE 2004. OIE Terrestrial Manual 2004, Bunyaviral Diseases of Animals. Updated: 23-07-2004.

16. Walker, J. S. 1975. Rift Valley Fever foreign animal disease. Their prevention, diagnosis and control.Committee of foreign animal disease of the United States. Animal Health Assoc., 209 – 221.
17. Reed, L.J and Muench, H. 1938. Simple method for estimating 50 percent end point. Amer. J. Hyg., 27: 493 – 497.
18. Paweska, J.T. ; Burt, F.J. ; Anthony, F.; Smith, S.J. ; Grobbelaar, A.A.; Groft, J.E.; Ksiazek, T.G. and Swanepoel, R. 2003.IgG . Sand wich and IgM Capture ewzy me Linked immunosorbent assay for the detection of antibody to Rift valley fever virus in domestic ruminants. J. virol. Methods, 113: 103-112.
19. Voller, A.; Bidwell, D. and Bartlett, A. 1976. Microplate enzyme immunoassay for the immunodignosis of virus infection. Am. Soc. Micro. 506-512.
20. Eduard, K. 1985. Progress in enzyme immunoassays: production of reagents, experimental design and interpretation. Bulletin of the world health organization, 63:793-811.
21. Marcoss, T.N.; Lily, S. Salama and Elian, A. Aly 1998. Studies of different adjuvants on the immune response of sheep to Rift Valley fever inactivated vaccine. Vet. Med. J., Giza, 64 (4B): 719-727.
22. Ibrahim, A. M. 2002. Improvement and evaluation of inactivated Rift Valley Fever vaccine, Ph. D. Thesis, Infectious Diseases, Fac. Vet. Med., Moshtohor, Zagazig Univ.
23. Derek T. O 'Hagan and Manamohan Singh 2007. MF59: A safe and potent oil-in-water emulsion adjuvant. A textbook "Vaccine adjuvant and delivery system".pp.115-129. Published: August 2007, John Wiley and Sons Ltd, USA.
24. 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: 293-299.
25. El Nimr, M.M. 1980. Studies on the inactivated vaccine against Rift Valley Fever. Ph. D. Thesis (Microbiology) Fac. Vet. Med. Assuit Univ. Egypt.
26. Hassan, K. E. Z. 1998. Comparative studies on inactivated and attenuated Rift Valley Fever vaccines. Ph. D. thesis (Infectious diseases) Fac. Vet. Med. Benha branch, Zagazig University, Egypt.
27. Gihan, K. M. 1990. Studies on Rift Valley Fever among animals in Egypt. Ph D Thesis, Inf. Dis., Fac. of Vet. Med., Zagazig Univ., Egypt.
28. El Karamany, R. M. 1981. Studies on production of Rift Valley Fever vaccine in tissue culture. Ph. D. Thesis (Microbiology), Fac. Vet. Med., Cairo Univ.
29. Iyer, A. V.; Ghosh, S.; Singh, S. N.; and Deshmukh, R. A. 2000. Evaluation of three "ready to formulate" oil adjuvants for FMD disease vaccine production. Vaccine, 19: 1097-105.
30. Patil, P.K; Bayry, J.;Ramkrishna. C.; Hygar, B.,; Misra, L.D. and Natarajan, C. 2002.Immune response of goat against FMD quadrivalent vaccine: Comparison of double oil emulsion and aluminum hydroxide gel vaccine,20:2781-2789.
31. Paweska, J.T.; Mortimer, E.; Leman, P.A. and Swanepoel, R. 2005. An Inhibition enzyme – linked immunosorbent assay for the detection of antibody to Rift valley fever virus in humans, domestic and wild ruminants. J. virol. Methods, 127: 10-18.
32. Catherine Ceˆtre-Sossah, Agne` s Billecocq, Renaud Lancelot, Ce´ dric Defernez, Jacques Favre, Miche` le Bouloy, Dominique Martinez, Emmanuel Albina 2009. Evaluation of a commercial competitive ELISA for the detection of antibodies to Rift Valley fever virus in sera of domestic ruminants in France. Preventive Veterinary Medicine Journal 90: 146–149.
33. Ali, S.M., Abdel Baky, M.H. and Al-Blowi, M.H. 2012. Comparison Between two Commercial Kits used for Detection of Anti-Rift Valley Fever Antibodies in Sheep Vaccinated with Smithburn Vaccine, Short Communications. Journal of Advanced Veterinary Research, Vol 2, No. (3): 208 – 210.

تقييم لقاح حمى الوادي المتصدع المثبط الممتزج مع زيت البارافين

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الملخص العربي

حمى الوادي المتصدع مرض حاد يسبب الحمى في المجترات ويسببه فيروس ينتقل بواسطة البعوض ينتمي إلى جنس Phlebovirus، عائلة الفيروسات Bunyaviridae، يتم تطعيم الحيوانات القابلة للإصابة بالمرض في مصر باستخدام لقاح حمى الوادي المتصدع المثبط والمحضر محليا مع ممتزج جل هيدروكسيد الألومنيوم. يهدف البحث إلى تحضير لقاح جديد لحمى الوادي المتصدع باستخدام ممتزج زيت البارافين باستخدام صيغة (W / O / W) . تم تقييم الاستجابة المناعية للقاح المحضر في الاغنام مقارنة مع لقاح حمى الوادي المتصدع المثبط والمحضر محليا مع ممتزج جل الألومنيوم هيدروكسيد وذلك من خلال استخدام اختباري المصل المتعادل والانزيم المدمص المناعي إليزا. أعطت النتائج الأفضلية للقاح حمى الوادي المتصدع الممتزج مع زيت البارافين بسبب ارتفاع درجة التعزيز المناعي ودون سمية ومع مدى أطول لمدة المناعة التي امتدت حتى 9 أشهر.

(مجلة بنها للعلوم الطبية البيطرية: عدد 25(1):157–164, سبتمبر 2013)