**Clinical and immunological studies on live attenuated Rift Valley Fever vaccine**

Heba A. Khafagy1 ; Mohamed H. Ebied2, Mohamed G.Abdelwahab2 and Mohamed A. Saad1

1Central Laboratory for Evaluation of Veterinary Biologics, Abbassia, Cairo, Egypt.

2Department of Animal medicine, Faculty of Veterinary Medicine, Benha University

[H.zahra568@yahoo.com](mailto:H.zahra568@yahoo.com)

**Abstract:** Addition 0.2 ug/ml of Queel A to RVF vaccine as adjuvant and inactivator on live RVF vaccine at the time of vaccination was studied. Safety and sterility of the prepared vaccine was ensured then its potency was evaluated in vaccinated sheep using SNT. Humeral immune response to the prepared vaccine was evaluated in sheep and compared with inactivated RVF vaccine and live attenuated smithburn RVF vaccine. Protective serum neutralizing antibody titer of prepared vaccine started at three weeks post vaccination and reach to the peak at five months then give last protective level at ten months but inactivated RVF vaccine give protective level after three weeks post vaccination and reach to the peak at three months, then give last protective level at seven months. Live attenuated smithburn RVF vaccine give protective level after two weeks post vaccination then reach to the peak at four months but it wasn't safe at pregnant animals causing abortion and teratogenic effect. It was concluded that prepared vaccinewas safe, sterile, potent and give high and long duration of immunity.

[Heba A. Khafagy; Mohamed H. Ebied, Mohamed G.Abdelwahab and Mohamed A. Saad. **Clinical and immunological studies on live attenuated Rift Valley Fever vaccine.** *N Y Sci J* 2013;6(6):12-17]. (ISSN: 1554-0200). <http://www.sciencepub.net/newyork>. 2

**Key words**: Queel A, RVF, Vaccine, Immunology

**1. Introduction:**

Rift Valley Fever (RVF) is one of the most important arthropod born viral diseases in Africa, primary affecting domestic animals with occasional involvement of man (**Daubney,*et al.,* 1931 ;Easterday, 1965 and Meegan, 1981).**The disease is most severe in sheep, cattle and goats causing high mortalities and abortion in pregnant animals, it runs in a rapid course with short incubation period (**OIE, 2001).**It caused by Bunya virus of the genus Phlebo virus and transmitted by mosquitoes (**OIE, 1989).** It was recorded in Rift Valley area in Kenya 1931 as described by (**Daubney,*et al.,* 1931).** Since then many authors reported the occurrence of the disease in different parts in African countries as Uganda (**Smith burn, 1949).**South West Africa and reached Sudan in 1973 (**WHO, 1978).**

In 1977 – 1978 an epidemic of RVF were recorded in Egypt as an acute febrile like illness with rigors, mylagia, headache, conjunctivitis and nausea with some ocular complications (**ElAkkad,1978 and Imam,*et al.,* 1978).**

Control of RVF disease in Egypt depends mainly on vector control and vaccination (**Abd El Ghafar,*et al.,* 1979).** So many trials for preparation of either live attenuated or inactivated vaccines were carried out beginning early with the first outbreak (**Abdel-Ghaffar, *et al.,*1979)*.*** and extended until now to reach to the most potent and safe vaccine from the local isolated strains (**Abou-Elfadl, 2007).**The progress in vaccine production is directed towards the selection of the proper adjuvant that can elaborate high and long standing immunity. Adjuvants considered one of the important factors in vaccine formulations that increase the immune response either humeral or cell mediated immunity (**Dalsgaard, 1990).**

**Black, 1977,** indicated that Queel A was more efficient adjuvant than aluminum hydroxide gel where antibodies are higher when Queel A was used. In addition Queel A based adjuvants have the ability to modulate the cell mediated immune system as well as to enhance antibody production and have the advantage that only a low dose is needed for adjuvant activity as stated by **Oda, 2000 and Marciani, 2003.**

The present work was planned to investigate the effect of addition of 0.2ug ugmlQueel A to live RVF vaccine before vaccination by one hour (prepared vaccine.),Estimation of immune response to this prepared vaccine, comparison the immune response of prepared vaccine, inactivated, live attenuated smithburn RVF vaccine, safety test of prepared vaccine at newly born lamb and pregnant ewes, detection of IgM in sera of vaccinated sheep by different types of vaccine by using ELISA and detection of virus shedding post vaccination by using ELISA.

**2. Materials and Methods**

**2.1. Virus:**

The original virus was that isolated from a human patient in Zagazig, Sharquia province and was supplied by NAMRU-3 after being identified to be RVF virus. It was obtained from RVF department, Veterinary Serum and Vaccine Research Institute, Abbasia Cairo with a final titre107.5 TCID50 / ml. It was used in SNT. It was kept at -70˚C.

**2.2.Adjuvant:**

**Queel A:**

It was supplied by Sigma-AldrishLabochemikalien Gm6H; Germany under the Cat.No:16109; lot.No:71500

**2.3. Vaccines:**

**2.3.1.prepared vaccine:**

Queel A was prepared as watery solution in phosphate buffered saline (PBS) with concentration 0.2ug/ml according to **(Amoros*et al.,*1987**) and sterilized by autoclaving. PBS with 0.2ug Queel A/ml was used as a diluent to the live RVF vaccine on the time of vaccination and injected directly S/C and I/P.

**2. 3.2. Inactivated RVF vaccine:-**

Tissue culture Binary inactivated RVF vaccine was prepared according to **(Eman , 1995).** It supplied by RVF vaccine department, Veterinary Serum & Vaccine Research Institute., Abbassia.

**2.3.3. Attenuated RVF vaccine (Smithburn strain):*-***

It was supplied by RVF Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

**2.4. Design of experiment :-**

Twenty adult sheep and 3 lambs .They purchased from El-Sharkia, Abou-Hamad, Elwaha farm. Sera of these animals were examined using serum neutralization test and enzyme linked immunosorbant assay to be sure that they are free from neutralizing antibodies against RVFV. While 2pregnant ewes and 2 lambs were inoculated with 10ml **(**5 ml S/C and 5ml I/P) used at safety test to prepared vaccine and one pregnant ewe and one lamb were kept as control. Seventeen adult sheep were divided into 4 groups.

**Group 1:** Composed of 5 animals were inoculated S/C with 1ml of prepared vaccine

**Group 2:** Composed of 5 animals were inoculated S/C with 1ml Inactivated RVF vaccine.

**Group 3:** Composed of 5 animals were inoculated S/C with 1ml of Live attenuated smithburn vaccine

**Group 4**: Composed of 2 sheep were kept as control.

The 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 vaccinated sheep weekly and stored at -20°C and inactivated at 56°C for 30 minutes before being used in the test.

**2.6. Serum Neutralization Test (SNT):-**

This test was used to detect the specific neutralizing antibodies against RVFV in the serum samples of vaccinated sheep according to method of constant serum- virus dilution procedure. The serum-neutralizing index was calculated according to **Reed and Muench, 1938 .**

**3.Results**

**3.1 Results of sterility test:**

Sterility test was carried out on the prepared vaccine gave satisfactory results. It was free from aerobic & anaerobic bacteria, fungi and mycoplasma.

**3.2. Results of safety test of prepared vaccine:**

Two susceptible pregnant ewes (one year old ) and two newly born lambs (7-10 day old)were inoculated with 10 ml of prepared vaccine (5ml S/C and 5ml I/P) and the 3rd of them kept as control. The animals observed for 14 days. They appeared healthy, did not show any clinical abnormalities,no rise of temperature as shown in **Table (1)**.

**3.3.Detection shedding of virus:**

The collected ocular, nasal and rectal swabs from sheep vaccinated with three types of vaccines were tested by ELISA for detection of shedding of RVF virus as shown in **Table (2).**

**3.4. Evaluation of the humoral immune response in sheep vaccinated with three types of RVF vaccines :**

**3.4.1.Group1 vaccinated with prepared vaccine**

The prepared vaccine give protective level (1.5) at the 21th day post vaccination and increased gradually till reach the peak (2.7) at 20 weeks post vaccination then the level decreased to be (1.5) at 40 weeks post vaccination ***.***

**3.4.2. Group 2 vaccinated with inactivated RVF vaccine**

The inactivated RVF vaccine give protective level (1.74) at the 21th day post vaccination and increased gradually till reached the peak (2.7) at 12 weeks post vaccination then the level decreased to be (1.56) at 28 weeks post vaccination.

**3.4.3. Group 3 vaccinated with live attenuated Smithburn RVF vaccine:-**

The live attenuated Smithburn RVF vaccine give protective level (1.56) at the 14th days post vaccination and increased gradually till reach the peak (3.5) at16 weeks post vaccination then it decline to (1.5) till the end of experiment .as shown in **Table (3), Figure (1)**

**5. Detection of IgM antibody by ELISA in sheep vaccinated by three types of vaccines:**

Detection of IgM in the serum of sheep vaccinated by three types of vaccines for 7 days post vaccination showed that absence of IgM in the serum of vaccinated Sheep in group 1 and group 2 while presence of IgM in the serum of vaccinated Sheep with live attenuated Smithburn RVF vaccine as shown ***in Table (4).***

**Table (1): Daily record of body temperature of pregnant ewes and new born lambs inoculated withprepared vaccine**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | **Days Post vaccination** | **Body Temperature** | | | | | | **Clinical signs** | | | | E1 | L1 | E2 | L2 | E3 | L3 | E1 , L1 | E2 , L2 | E3 , L3 | | **0** | *38.9* | *39.0* | 39.0 | 39.0 | *38.9* | *38.9* | ـــــ | ـــــ | ـــــ | | **1st** | *38.8* | *38.9* | 39.1 | 39.1 | *39.0* | *39.0* | ـــــ | ـــــ | ـــــ | | **2nd** | *39.0* | *38.9* | 39.2 | 39.2 | *39.4* | *39.5* | ـــــ | ـــــ | ـــــ | | **3rd** | *39.4* | *39.1* | 39.0 | 39.0 | *39.2* | *39.4* | ـــــ | ـــــ | ـــــ | | **4th** | *39.2* | *39.0* | 39.0 | 39.0 | *39.0* | *39.2* | ـــــ | ـــــ | ـــــ | | **5th** | *38.9* | *39.2* | 39.0 | 39.0 | *39.3* | *38.9* | ـــــ | ـــــ | ـــــ | | **6th** | *39.0* | *39.3* | 38.9 | 38.9 | *38.8* | *39.0* | ـــــ | ـــــ | ـــــ | | **7th** | *39.1* | *38.9* | 39.1 | 39.2 | *39.0* | *39.1* | ـــــ | ـــــ | ـــــ | | **8th** | *38.9* | *38.9* | 39.3 | 39.3 | *38.9* | *38.9* | ـــــ | ـــــ | ـــــ | | **9th** | *39.0* | *39.0* | 39.5 | 39.5 | *39.0* | *39.0* | ـــــ | ـــــ | ـــــ | | **10th** | *38.9* | *39.3* | 39.1 | 39.4 | *39.2* | *39.1* | ـــــ | ـــــ | ـــــ | | **11th** | *39.2* | *39.4* | 39.1 | 39.1 | *39.5* | *39.2* | ـــــ | ـــــ | ـــــ | | **12th** | *39.2* | *39.0* | 39.0 | 38.9 | *39.3* | *39.2* | ـــــ | ـــــ | ـــــ | | **13th** | *39.4* | *39.0* | 38.9 | 39.1 | *39.4* | *39.4* | ـــــ | ـــــ | ـــــ | | **14th** | *39.0* | *39.4* | 39.0 | 39.0 | *39.0* | *39.0* | ـــــ | ـــــ | ـــــ | |

(ـــــ) Absence of any abnormal clinical signs E: Pregnant ewe L: New born lamb

**Table (2):Detection of RVF virus in swabs using antigen detection ELISA.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Days**  **Post**  **vaccination** | **Antigen detection ELISA**  **for RVF virus in swabs** | | | | | | | | | | | | | | |
| **Sheep vaccinated with prepared vaccine** | | | | | **Sheep vaccinated with Inactivated RVF vaccine.** | | | | | **Sheep vaccinated with Live attenuated smithburnvaccine.** | | | | |
| **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** |
| **1** | - | - | - | - | - | - | - | - | - | - | + | + | + | + | + |
| **2** | - | - | - | - | - | - | - | - | - | - | + | + | + | + | + |
| **3** | - | - | - | - | - | - | - | - | - | - | + | + | + | + | + |
| **4** | - | - | - | - | - | - | - | - | - | - | + | + | + | + | + |
| **5** | - | - | - | - | - | - | - | - | - | - | + | + | + | + | + |
| **6** | - | - | - | - | - | - | - | - | - | - | + | + | + | + | + |
| **7** | - | - | - | - | - | - | - | - | - | - | + | + | + | + | + |
| **8** | - | - | - | - | - | - | - | - | - | - | + | + | + | + | + |
| **9** | - | - | - | - | - | - | - | - | - | - | + | + | + | + | + |
| **10** | - | - | - | - | - | - | - | - | - | - | + | + | + | + | + |
| **11** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| **12** | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

( - ): Not RVF virus detected. ( + ): Detected RVF virus.

**Table(3): Mean of neutralization index in vaccinated and non vaccinated groups:-**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | | Groups of animals | **SNT Titer\*** | | | | | | | | | | | | | | | | | | **Weeks post vaccination.** | | | | | | | | | | | | | | | | | | **0** | **1** | **2** | **3** | **4** | **6** | **8** | **12** | **16** | **20** | **24** | **28** | **32** | **36** | **40** | **44** | **48** | | **Group.1** | **0.5** | **0.9** | **1.2** | **1.5** | **1.74** | **1.92** | **2.0** | **2.2** | **2.6** | **2.7** | **2.52** | **2.2** | **2.0** | **1.8** | **1.5** | **1.32** | **0.9** | | **Group.2** | **0.5** | **0.9** | **1.26** | **1.74** | **1.9** | **2.1** | **2.52** | **2.7** | **2.3** | **2.2** | **1.8** | **1.56** | **1.26** | **0.9** | **0.72** | **0.54** | **0.48** | | **Group.3** | **0.5** | **0.9** | **1.56** | **2.0** | **2.3** | **2.7** | **2.9** | **3.4** | **3.5** | **3.3** | **2.8** | **2.5** | **2.4** | **2.3** | **2.04** | **1.8** | **1.5** | | **Group.4** | **0.35** | **0.25** | **0.25** | **0.3** | **0.35** | **0.35** | **0.3** | **0.35** | **0.3** | **0.3** | **0.2** | **0.3** | **0.35** | **0.34** | **0.25** | **0.3** | **0.3** | |

Group 1 :sheepvaccinated with prepared vaccine. Group 2: sheep vaccinated with Inactivated RVF vaccine .

Group 3 : sheep vaccinated with live attenuated smithburn vaccine. Group 4 : control non vaccinated sheep.

Protective antibody titer = 1.5 \*Log10 serum neutralizing antibody titer.

**Table(4) :-Detection of IgM by ELISA in the serum of sheep vaccinated with three types of vaccine.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Days post vaccination** | | | | | | | **Groups of animals** |
| **7th** | **6th** | **5th** | **4th** | **3rd** | **2nd** | **1st** |
| (-ve) | (-ve) | (-ve) | (-ve) | (-ve) | (-ve) | (-ve) | **Group.1** |
| (-ve) | (-ve) | (-ve) | (-ve) | (-ve) | (-ve) | (-ve) | **Group.2** |
| (+ve) | (+ve) | (+ve) | (+ve) | (+ve) | (+ve) | (+ve) | **Group.3** |
| (-ve) | (-ve) | (-ve) | (-ve) | (-ve) | (-ve) | (-ve) | **Group.4** |

Group 1:sheepvaccinated with prepared vaccine.Group 2: sheep vaccinated with Inactivated RVF vaccine .

Group 3: sheep vaccinated with live attenuated smithburn vaccine. Group 4: control non vaccinated sheep.

+ve : detected IgM. -ve : no IgM detected.

**4. Discussion**

Adjuvants are considered one of the important factors in vaccine formulation so, the progress in vaccine production is directed towards selection of the proper adjuvant that can elaborate high and long lasting immunity (**Dalsgaard, 1990)**.Queel A is one of these agents studying its effect on the live vaccine as inactivator used at the time of vaccination as well as its effect on the immune response of vaccinated animals.

This study is applied to evaluate the immune response and duration of immunity in sheep vaccinated with the prepared vaccine in comparison to different kind of vaccines (inactivated , live attenuated Smithburn RVF vaccine).

The prepared vaccine was proven sterile and free from bacterial and fungal contamination. These result agreed with that of **Schipper and kelling (1975)** and goes along with **(Wassel*et al.* 1996)** and the **Code of Federal Regulations (2005),** who reported that the final product should be free from bacteria, fungi and mycoplasma. Safety of the prepared vaccine in pregnant ewes and newly born lambs (5 ml S/C , 5 ml I/P ) gave satisfactory results of safety with no rise in body temperature. It remained within the normal range for 14 days post vaccination and no clinical abnormalities .It is safe in pregnant animals and newly born lambs as it has no any teratogenic or aborteogenic effect.

**Table (2)** showed that there was no evidence to virus shedding through nasal, ocular or rectal swabs in sheep vaccinated with prepared vaccine or inactivated vaccine the obtained result agree with **Eman, 1990*.*** But ***s***heep vaccinated with live attenuated smithburn RVF vaccine indicated shedding of the virus at short time due to presence of smithburn RVF virus in blood to short time give chance to transportation of it by insects from animal to other. So, the prepared vaccine is safe from this point.

**Table (3)** indicated that the humeral immune response to the prepared vaccine started at three weeks post vaccination ,reach to the peak at five months and give last protective level at ten months but inactivated RVF vaccine give protective level after three weeks post vaccination ,reach to the peak at three months and give last protective level at seven months. Live attenuated smithburn RVF vaccine give protective level after two weeks post vaccination ,reach to the peak at four months but it wasn't safe at lambs and pregnant animals causing abortion and teratogenic effect .

**Table (4)** showed that absence of IgM in animals vaccinated with prepared and inactivated vaccine and this indicated complete inactivation of prepared vaccine , while presence of IgM in the serum of vaccinated sheep with live attenuated Smithburn RVF vaccine .

From the above studies the prepared vaccine is sterile, safe, potent and give high and long duration immunity.

So addition of Queel A to live RVF vaccine could be recommended as safe inactivator and immune modulator providing high and long duration immunity.

**References**

1. **Abdel Gaffar, S.; Mohsen, A.; Ayoub, N.; El-Nimr, M. and Fathia, M. (1979):**Rift Valley fever in Egypt III. Diagnosis andvaccination.14th Arab. Vet. Cong., 24-29 March Cairo, page 1-11.
2. **Aboul-Fadl, G. (2007):** Trial for production of improved inactivated RVFV vaccine. M.V.Sc. Thesis, Virology. Faculty of Veterinary Medicine, Cairo University.
3. **Amoros, M.; Fauconnier ,B. And Girre, R.L. (1987):***In vitro* antiviral activity of queel A from Anagallisarvensis ,primulaceae, against herps simplex virus and polio virus.
4. **Black, L. (1977):**FMD vaccination Vet .Rec., 100 (10): 195-198.
5. **Code of Federal Regulation (2005):** Animal and animal products 9, 2005. Published by the Office of the Federal Register National Archives and Record , Administration. Vet. Microbial.,6(2):101-107.
6. **Dalsgarrd,K.;Hilgers,L. and Trouve,G. (1990):**Classical and new approaches to adjuvant use in domestic food animals; Adv.Vet. Sci. Comp. Med., 35: 121-159.
7. **Daubney, R.; Hudson, J.R. and Graham, P.C. (1931):** Rift Valley Fever or Enzootic hepatitis as undescribed diseases of sheep, cattle and man from East Africa. J. Path. Bact., 34 (1): 545 - 579.
8. **Easterday, B. C. (1965):** Rift Valley Fever. In advances in Veterinary ScienceEds. C. A. Brandly and C. Cornelius, eds Academic press (New York), Vol, 10, p. (65): 127.
9. **El Akkad, A. M. (1978):** Rift Valley Fever outbreak in Egypt, October-December 1977.J. of the Egypt. Public Health Assoc. Vol. L III (3 and 4): 123-12.
10. **Eman, M. S. (1990):**Studies on validity of Rift Valley Fever vaccine. M. V. Sc. Thesis, Vet. Microbiology, Fac. Vet. Med., Alex. Univ.
11. **Eman, M. S. (1995):** Studies on Rift Valley Fever vaccine inactivated with Binary. Ph. D. Thesis, Microbiology, Fac. of Vet. Med., Cairo Univ.
12. **Imam, Z. E. I.; El Karamany, R. E. and Darwish, M. A. (1978):**An epidemic of Rift Valley Fever in Egypt. Isolation of RVF virus from animals. J. of the Egyptian Public Health Assoc., 11 (3, 4): 256 – 259.
13. **MarcianD.j. (2003):** Vaccine adjuvant : Role and mechanisms of action in vaccine immunogenicity. Drug Discov. Today, Oct. 15: 8(20) :934-43.
14. **Meegan, J.M. (1981):** Rift Valley fever in Egypt: An overview of the epizootics in 1977and 1978. Contrib. EpidemiolBiostat. 3, 100-103.
15. **Oda, R.j. (2000):**Adjuvant and haemolytic activates of 47 saponines derived from medicinal and food plants .Biol.Chem.,381(1) :67-74.
16. **O.I.E. (1989):**Manual of recommended diagnostic techniques and requirments for biological products of lists A and B diseases. International Committee of the OIE, Vol. 2 P. 1/8 - 8/8.
17. **O.I.E. (2001):**Rift Valley Fever.
18. **Reed, L.J. and Muench, H. (1938):**Simple method for estimating 50 percent end point. Amer. J. Hyg., 27: 493 - 497.
19. **Schipper, I.A. and Kelling, C.L. (1975)**: Evaluation of an inactivated IBR vaccine. Canada. J. of Comp. Med., 39(4): 402-406.
20. **Smithburn, K. C. (1949):**Rift Valley Fever. The neurotropic adaptation of virus and experimental use of this modified virus as a vaccine, Br. J. Exp. Path., 30: 1 – 16.
21. **Wassel, M.S.; AboulSaoud, S.; Girgis, S.; Hussein, A.Z. and Eman, El-Rawy (1996):** Trials for preparation and evaluation of acombined vaccine for IBR (IBR-V), (BVD-V), (PI-3V), *P. multocida* and *P. haemolytica* in Egypt. Assiut Vet. Med. J., 35 (70).
22. **WHO (1978):**Rift Valley Fever. A Review. Bull. Epiz. Dis. Afr., 5: 431-458.

3/2/2013