

## RESEARCH ARTICLE

# Correlation Between Rift Valley Fever Virus (RVFV) Neutralizing Antibody Titers in Vaccinated Sheep and Effective Dose 50 (ED<sub>50</sub>) in Vaccinated Mice

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## ABSTRACT

Rift valley fever is an arthropod-born, multispecies zoonotic viral disease. Control of RVF disease depends mainly on vector control and vaccination of susceptible animals. The present work aims to detect the correlation between Rift Valley Fever Virus (RVFV) neutralizing antibody titers in vaccinated sheep using Serum Neutralization test as *in vitro* test and effective dose fifty in vaccinated mice as *in vivo* potency test and determine if they can be alternative to each other. In this work, 17 inactivated RVFV vaccine batches were evaluated, applying SNT for serum samples of vaccinated sheep and ED<sub>50</sub> in vaccinated mice. The two models of tests showed compatible results, where the same 14 vaccine batches showed satisfactory results [(SNT >1.5) and (ED<sub>50</sub> <0.02)], while the other three batches revealed unsatisfactory results in both two tests. Statistical analysis of results using Wilcoxon's test was (0.0001), indicating a significant correlation between the tests so it could be recommended to depend on SNT instead of mice inoculation in the evaluation of RVF vaccine to reduce the numbers of animals being used and to avoid the possible public health hazard.

**Keywords:** Effective Dose Fifty, Inactivated Rift Valley Fever vaccine, Mice, Serum Neutralization Test, Rift Valley Fever, Sheep.

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## INTRODUCTION

Rift Valley Fever (RVF) is an arthropod born viral disease of sheep, cattle, and goats (Mona, 2000; FAO, 2004). It is caused by Bunyavirus of the genus Phlebovirus and transmitted by mosquitoes (OIE, 2018). It was first recorded in Kenya in 1931 Daubney *et al.* (1931) since then many outbreaks had been reported in different parts of African countries (Uganda and southwest Africa), as well as it also appeared in Saudi Arabia and Yemen (FAO, 2003). In Egypt, it was introduced as an epidemic disease in 1977, followed by in 1978, 1993, 1997, and 2003 (Mohamed Kenawy *et al.*, 2018). RVF is a zoonotic disease with the short incubation period, causing high mortalities, abortion in pregnant ewes, and fetal malformation in ruminants. At the same time, it causes hemorrhagic fever, neurological disorders and blindness in humans (Indran and Ikegami, 2012). Control of RVF disease depends mainly on vector control and vaccination (Abdel Ghaffar *et al.*, 1979). There are two types of RVF vaccines, live Smithburn Rift valley fever vaccine and Inactivated Rift valley fever vaccine, the inactivated RVF vaccine mostly used in the field as it is safer than the live one. In Egypt, it is produced by Veterinary Serum and Vaccine Research Institute (VSVRI) and evaluated at Central Laboratory for Evaluation of Veterinary Biologics (CLEVB) by estimation antibody titer in sheep using Serum Neutralizing test (SNT) (OIE, 2017) and Challenge test in Mice. The aim of the present study was to find correlation between Rift valley fever virus neutralizing antibody titres in vaccinated sheep using SNT *in vitro* test and ED<sub>50</sub> *in vivo* test in mice.

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## MATERIAL AND METHODS

For serum neutralization test and ED<sub>50</sub> potency test the necessary animals and other requirements were obtained as below.

Seventeen batches of local commercial inactivated RVF vaccines were delivered to Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), Abbassia-Cairo. These batches had been evaluated around the last five years for sterility, safety, and potency tests.

Strain Bank supplied African green monkey kidney cells (Vero) at CLEVB. Cells were grown and maintained, according to Macpherson and Stocher (1962). The cells were used for the serum neutralization test (SNT).

Seventy adult Swiss albino mice (21-35 days old) were available by CLEVB for each batch and used for potency ED<sub>50</sub> test (fifty mice for vaccine dilutions, ten mice for positive control, and ten mice for a negative control).

Five adult sheep of native breed (six months old) were provided by CLEVB, free from any antibodies against RVFV for each batch. They were used for evaluation of inactivated Rift Valley Fever vaccine batches by inoculation each sheep with field dose of the vaccine subcutaneously. Blood samples were collected from vaccinated sheep at 28<sup>th</sup> days post-vaccination then tested by serum neutralization test.

Rift Valley Fever virus strain (code RVF/ Sharqia/1977, Genotype ZH-501-777Seg-M DQ3802001) was obtained from Strain Bank at CLEVB. This virus was used in SNT (as 100 TCID<sub>50</sub>), also it was adapted and propagated in mice to be used in ED<sub>50</sub> test (as 10<sup>3</sup>-10<sup>4</sup> MIPLD<sub>50</sub>) in mice.

### Serum neutralization test

It measures the humoral immune response against RVF Virus for sera of vaccinated sheep by inactivated RVF vaccine batches, the test was performed by using the microtechnique as described by Walker (1975), and serum-neutralizing titer was calculated according to Reed, and Muench (1938) and was expressed as log<sub>10</sub> TCID<sub>50</sub>/mL.

### ED<sub>50</sub> potency test

Five-fold dilutions of RVF vaccine were prepared in suitable media starting from 1:1 to 1:625. Ten mice were inoculated

with each dilution, 0.2 ml of the diluted vaccine was inoculated intra potential as a first dose followed by a second dose one week later. Seven days after the second inoculation, all mice were challenged via the I/P route with 0.1 ml. RVFV containing 10<sup>3</sup>-10<sup>4</sup> MIPLD<sub>50</sub>/mL (Randall *et al.* 1964; CLEVB 2017). This is in addition to another two groups of mice; one group was inoculated with challenge virus as positive control and other group kept as a non-vaccinated non-challenged negative control. All groups of mice were kept under observation for 21 days where deaths were recorded daily. The ED<sub>50</sub>/ml was calculated according to the method of Reed and Muench (1938).

### Data analysis

The results of SNT of vaccinated sheep and ED<sub>50</sub> in mice for the 17 batches of inactivated RVF vaccine were statistically analyzed by Wilcoxon's test and compared.

## RESULTS AND DISCUSSION

The humoral immune response of vaccinated sheep using SNT for the 17 batches of inactivated RVF vaccine was determined, and the average of antibody titer was expressed as log<sub>10</sub> TCID<sub>50</sub>/mL. The results of SNT showed that 14 batches reached protective neutralizing serum antibody titer (> 1.5), while the other three batches (3-8-11) didn't reach the protective titer (Fig. 1).

The results of ED<sub>50</sub> in mice for the same 17 vaccine batches showed that 14 batches of mice reached to the permissible

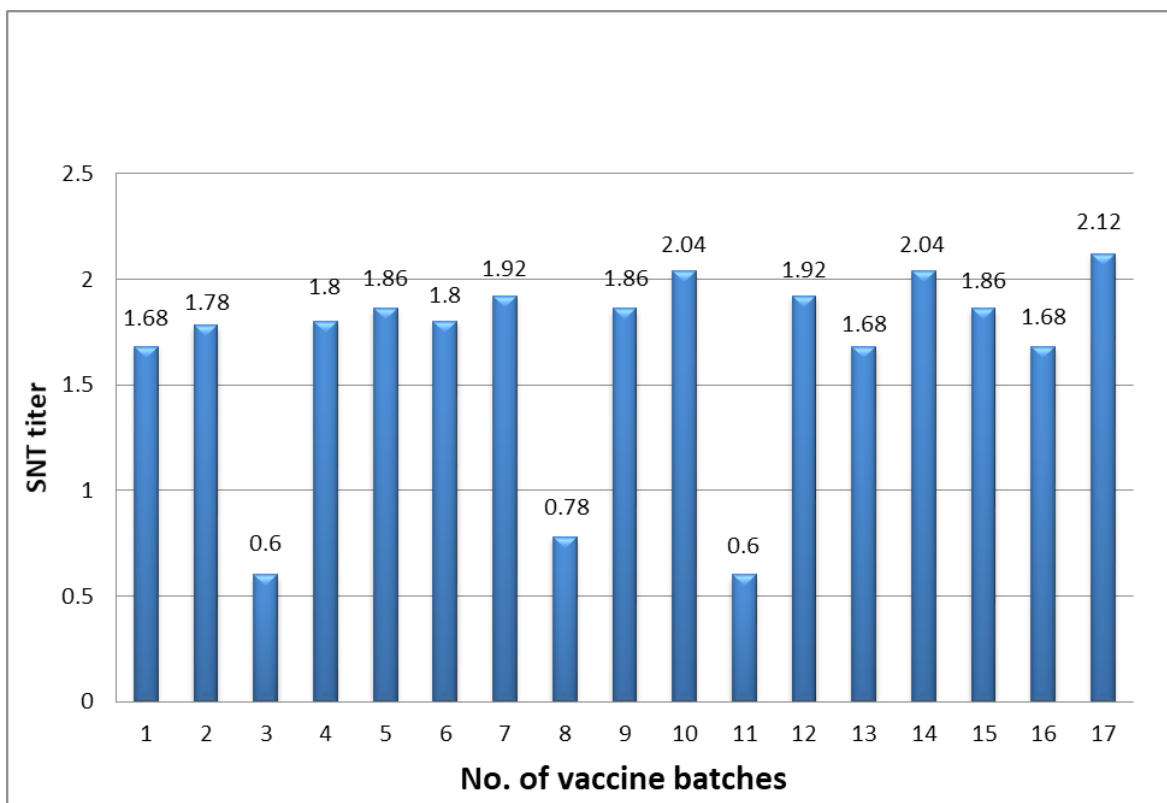


Fig. 1: Serum neutralizing antibody titers of vaccinated sheep by inactivated RVF vaccines

limit of  $ED_{50}$  ( $< 0.02$  ml), while the other three groups (3-8-11) didn't reach the permissible limit (Fig. 2).

Statistical data analysis of the results for the two tests using Wilcoxon's test showed a significant correlation (0.0001) ( $p < 0.05$ ).

Rift Valley fever virus (RVFV; Bunyaviridae: Phlebovirus) is primarily transmitted by mosquitoes and causes a potentially severe disease among both humans and animals. Primarily vaccination of susceptible animal and vector controlling are important measures to protect the livestock from the disease (FAO, 2014).

The live attenuated (Smithburn strain) vaccine reported unsafe which is restricted in use because it causes abortion to pregnant ewe, while inactivated RVF vaccine considered safe which is effective and widely used in the field, but it is more expensive, and at least two doses must be given (WHO/FAO, 1983).

The results show that the same 14 batches reached the protective level in both two tests. The permissible limit for SNT is ( $> 1.5 \log^{10} TCID_{50}$ ) as has been suggested by (Randall *et al*, 1964 and CLEVB, 2017) and these results agreed with (El-Bagoury *et al*, 2017 and 2013) who recorded that the protective antibody titer obtained by the inactivated RVF virus vaccines are more than 1.5, and for vaccine potency test ( $ED_{50}$ ) is  $< 0.02$ /mL in mice as recommended by WHO and also reported by Ebtesam *et al.*, (2016) and Maha *et al.*, (2017) who used  $ED_{50}$  test in evaluation and detect potent inactivated RVF vaccines prepared by different inactivates

and adjuvant which are less than 0.02. On the other hand, the same three batches not reached the protective level in both two tests (Figures 1 and 2), which reflect that the results of the two tests are compatible with each other.

Biostatistician tools are important tools in the analysis of data, so in this work, the two tests results (SNT in sheep and  $ED_{50}$  in mice) were analyzed using Wilcoxon's test, and gave 0.0001, which represent a significant correlation between the two tests results ( $P < 0.05$ ) (Elise and Jonathon, 2002).

Recently, the use of an *in vitro* method (as serological tests) was intended to replace the *in vivo* method (as  $ED_{50}$  mouse potency test and other challenge test), and that may provide an opportunity to reduce the numbers of animals being used in order to keep environmental harmony (Coenraad Hendriksen *et al.*, 1998), as  $ED_{50}$  test need a large number of mice (seventy mice for evaluation each batch) in comparison to less number of sheep used in SNT, as well as to reduce contact and manipulation with challenge virus.

## CONCLUSION AND RECOMMENDATION

There is a significant correlation between results of SNT in sheep vaccinated by inactivated RVF vaccine and results of  $ED_{50}$  in mice for the same vaccine batches. So it is recommended to depend only on SNT for vaccinated sheep as a serological *in vitro* test for the evaluation of inactivated RVF vaccine. In contrast, potency tests in mice ( $ED_{50}$ ) should be restrictedly conducted only in cases of new vaccine products for registration or if there are any changes in the

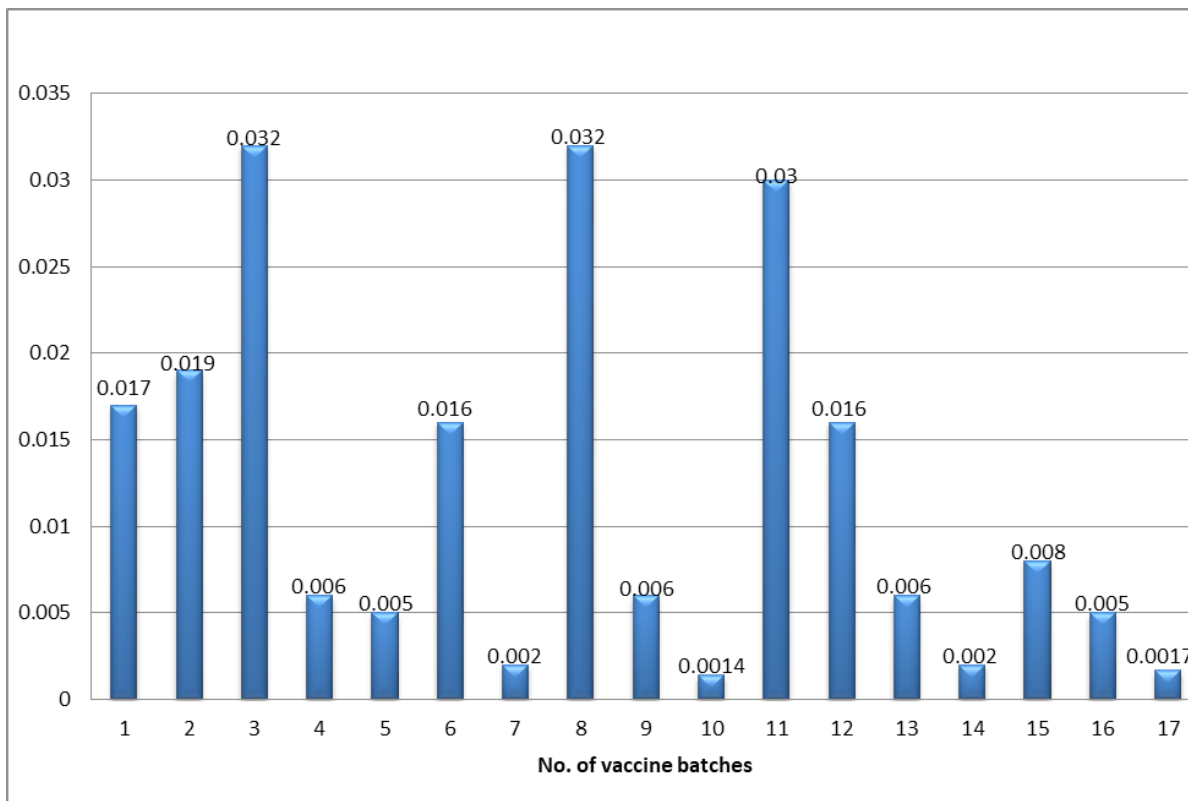


Fig. 2:  $ED_{50}$  in vaccinated mice by inactivated RVF vaccine

original vaccine product and from one period to another for a limited number of supplied batches.

Institutional Animal Care and Use Committee at Central Laboratory for Evaluation of Veterinary Biologics: hereby acknowledge the research manuscript, and it has been reviewed under our research authority and is deemed compliance to bioethical standards in good faith.

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