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# Response of laboratory staff to vaccination with an inactivated rift valley fever vaccine – TSI- GSD 200

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**Summary**
Laboratory staff and students were vaccinated with a formalin-inactivated rift valley fever (RVF) vaccine. This study showed that the vaccine used (TSI-GSD 200) was able to bring about the production of antibodies in recipients. For the production of a high titered antibody response, three doses of the vaccine were required. One or two doses of the vaccine did not produce a greater than four-fold rise in antibody titre. A greater than four-fold rise in antibody titre following vaccination, is considered significant. The complete dose of the vaccine, that is, three doses, was necessary for protection. This study also showed that the haemagglutination inhibition (HI) test was capable of detecting antibodies, few weeks post vaccination. Though such HI antibodies broaden with time, it could be used for screening purposes and a more specific test, e.g., plaque reduction neutralisation (PRN) test used for confirmation of such results.

**Keywords**: *Rift valley fever vaccine, laboratory staff*

**Résumé**
Les travailleurs de laboratoire et les etudients avaient ete vaccine avec la vaccin deontre la fieve de la valle du Rift fait sur la base de la formaline – inactive (RVF). Cette etude avait demontre que le vaccin utilise (TSI-GSD 200) avait ete capble de produire des anticorps chez les receveurs. Pour la production des anticorps a for titre, 3 doses de vaccin etait requis. Une on deux doses de vaccins n'avait pas produit un taux 4 fis plus eleves de titres d'anticorps chez les receveurs. Un titre d'anticorps superieur a 4 fois le titre normal est conodere significatif. La dore complete de la vaccination (3 doses) avait ete trouve necessaire pour confere une protection. Cettle etude avait montre que le tests d'inhibition de l'haemaglutination (IH) etait capable de detectr les anticorps, quelque remains apres la vaccination. Quoique les pareuls anticorps (IH) saccroissent avec du temps, il pourrait etre utilise pour les procedes de detectate. Un teste plus specifique e.g. le test de la reduction de neutralization sur plaque (PRN) pourrait etre utilise pour la confirmation de pareil resultats.

## Introduction

Rift valley fever (RVF) virus was initially an ungrouped arthropod-borne virus isolated in Africa [1]. RVF virus was recognized to posses high epizootic potential in the early 1930's in Kenya [2]. In the early 1950s in South Africa, there was an outbreak [3] and successive outbreaks followed in the 1960s and 1970s. These outbreaks were generated from persistent endemic foci initially limited to sub-Saharan Africa [4]. There was a massive epizootic in Egypt in 1977 and 1978 [5]. RVF was originally a disease of cattle and sheep [6]. In the 1931 Kenya incidence, the disease in man was described as an influenza-like disease occurring in animal handlers [2,7,8]. Human deaths resulted following natural RVF infections in South Africa in 1975 [9]. In 1979, there was a fatal incidence in Egypt resulting in a high rate of human involvement [5]. The clinical disease in humans includes sudden onset of malaise, fever, rigour, severe headache, back-breaking myalgia and anorexia [10]. The number and severity of human and animal cases and the limited ability to control such an extensive outbreak has long indicated the need for preventive measures. In Nigeria, the presence of RVF virus antibody has been demonstrated in domestic animals [11]. The existence of predisposing factors for a severe outbreak of RVF virus disease has been shown in Nigeria [12]. It is for this purpose that this work was carried out to determine the response of high risk individuals to vaccination with a formalin inactivated RVF vaccine in our environment.

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## Materials and methods

*Vaccine* A lyophilised formalin-inactivated RVF vaccine (TSI-GSD 200) was used. The-entebe strain of RVF virus was used and prepared in a diploid line of fetal rhesus monkey lung (FRhL-2) cells propagated in appropriate quantities of growth medium. For reconstitution, 5.5m of sterile injection water was used per vial. The dose given was 1 ml subcutaneously. The vaccine was provided by C.J. Peters of the United State Army Medical Research Institute for Diseases, Fort Detrich, Frederich, Maryland. The vaccination and bleeding schedule is as shown on Table 1.

**Table 1**: Schedule of vaccination and bleeding

| Day | Collection of blood sample | Vaccination |
|---|---|---|
| 0 | Pre-vaccination blood Sample collection | 1ml subcutaneously/ person |
| 14 | - | 1 ml subcutaneously/person |
| 28 | - | 1 ml subcutaneously/person |
| 42 | Post-vaccination blood Sample collected | - |

*Virus* A mouse brain passage of the Dakar strain of RVF virus ZINGA / (Dakar B. 1976) isolated from *Mansonia africanus* was used in this study.

*Blood* Blood samples were collected from staff members and students of the Virology Department, College of Medicine, University College Hospital, Ibadan and a few staff of the Postgraduate Institute for Medical Research and Training (Table 2). The blood was collected by venipuncture and allowed to clot at 4 °C overnight. The following day, the samples were centrifuged at 1,500 rpm for 10 minutes and the supernatant collected and stored at –20 °C.

**Table 2 :** Schedule of virus and sarcoma 180/Tg-cell administration

| Day | Virus | Vaccination |
|---|---|---|
| 0 | 0.2 ml/mouse ip inactivated virus and adjuvant mixture | |
| 7 | 0.2 ml/mouse ip inactivated virus and adjuvant mixture | |
| 14 | 0.2 ml/mouse ip live virus and adjuvant | |
| 19 | | 0.2 ml/mouse ip |
| 21 | 0.2 ml/mouse ip live virus and adjuvant | |

*Virus isolation in mice*
Virus isolation studies were performed in groups of 2-4 days old mice. A 10% suspension of infected mouse brain was inoculated intracerebrally into a group of two to three days old mice at a dose level of 0.2 l/mouse.Inoculated mice were observed for 2-3 days and mice that were ill were collected. The virus was passaged five times to build up the titer in the mice. Mice brains were finally harvested and pooled into a sterile universal bottle. The $LD_{50}$ was calculated according to the method of Reed and Muench as described by [13]

*Antigen production*
Sucrose antigen production was prepared as described by [14] This antigen was used in the haemagglutination and haemagglutination inhibition tests.

*Immune mice ascitic fluid (Imaf)*
RVF virus is lethal for adult mice therefore was inactivated as described in [15]. 0.25% of 10% of industrial formaldehyde was added to a known amount of $10^{-1}$ of the virus suspension, shaken and left at 4 °C for 3 days. To prepare the IMAF against RVF virus, adult mice were infected intraperitoneally with the formalin inactivated virus mixed with Freunds adjuvant. IMAF was raised with four intraperitoneal injections of the virus mixture as shown in Table 2. Twelve days after the administration of the last dose, the ascitic fluid was tapped. The tapped fluid was lightly centrifuged to remove cells and the supernatant fluid containing antibody was stored in 0.1 ml aliquots at -70°C until tested.

*Serological tests*
Sera samples were tested by HI after kaolin adsorption. This method was used as described by [14] adapted to microtiter plates. Materials for PRN tests were not available in the laboratory. Same samples were sent to the United States Army Medical Research Institute of Diseases, Fort Detrich, Maryland, where PRN test was performed. Serum samples, which were positive from the screening test, were assayed to determine the antibody titre. The antibody titre was considered to be the reciprocal of the highest serum dilution causing complete inhibition of haemagglutination.

## Results

Thirty-three paired samples were collected from vaccinees. Twenty-nine of these received three doses of the vaccine, 1 person received 2 doses and 3 persons received 1 dose of the vaccine, respectively.

*Hi Test*
The results of the HI test are shown in Table 3. Thirty of the 33 vaccinees seroconverted with a double to greater than four fold increase in antibody titre post vaccination. Two people showed no change in pre-vaccination antibody titre One person showed a drop in pre-vaccination antibody titre.

*Prn test*
The results of the PRN test are shown in Table 4. Twenty-five of the 28 vaccinees seroconverted with a double to greater than four fold increase in antibody titre post vaccination. Two people showed no change in pre-vaccination antibody titre. 1 person showed a drop in pre-vaccination antibody titre.

*Side Effects*
Reported side effects occurred within 24 hrs of vaccination and lasted for 24-48 hrs. This included body weakness (2.6%), mild fever (5.1%), body pain (5.1%) and localised swelling at the site of injection (2.6%). (Table 5).

## Discussion

Ninety-two percent of vaccinees who received three doses of the vaccine seroconverted and 88.9% of vaccinees who received less than three doses of the vaccine seroconverted with an insignificant rise in antibody titre. This is in line with the study of [16] where the smallest dose level of the RVF virus vaccine gave the lowest geometric mean titre, but was not completely devoid of antigenicity. This indicates that the effectiveness of the vaccine depends on the dose given. This dose response is necessary since there has to be adequate amount of antigen present in the body to induce and maintain an immune response. Some vaccinees who received the complete dose of the vaccine did not seroconvert. Absence of seroconversion following administration of complete dosage of a vaccine could be due to the low potency of the vaccine or individual factors such as state of the individual's immune system, presence of other disease causing organisms, environmental and nutritional status and the general condition of the individual [19]. Potency of vaccine is not the case in this study as evidenced by the percentage of vaccinee that showed significant seroconversion. A vaccine recorded a drop in antibody titre post vaccination This can be attributed to the presence of RVF virus antibody in the individual. On introduction of the same antigen, the existing antibody mops up the incoming antigen before it could elicit an immunological response. In this case, booster dose is required to increase the antibody health titre. Of the 17 people who were vaccinated three years prior, only 3 people had an antibody titre of about 10 at the beginning of this study. Though [8] in 1947 recorded a persistence of RVF virus neutralising antibody after 12 years following infection, this study records an 82.5% loss in antibody titre in a period of three years. However, the kinectics of antibody developed three years prior was not studied to find out if the dose given at that time was acting as a booster dose. The differences could be attributed to the fact that [8] were discussing a natural infection, which acts as a live vaccine, therefore, the immunity lasts longer. This study, however, is dealing with an inactiviated vaccine. [16] also found this vaccine to be characterised by short-lived antibody response with a steep

**Table 3:** Haemagglutination inhibition antibody response of 33 laboratory staff members receiving different doses of RVF virus vaccination

| | | Post vaccination HI antibody status | | | |
|---|---|---|---|---|---|
| Number of doses | Pre-vaccination HI antibody status | >4 fold rise | <4 fold rise | No change | Decrease |
| 3 | Negative 19 | 19 | - | - | - |
| | Positive 10 | 6 | 1 | 2 | 1<br>80-160 |
| 2 | Negative - | - | - | - | - |
| | Positive 1 | | 1 | - | - |
| 1 | Negative 2 | - | 2 | - | - |
| | Positive 1 | - | 1 | - | - |
| Total | 33 | 25 | 5 | 2 | 1 |

**Table 4:** Plaque reduction neutralising antibody response of 28 laboratory staff members receiving different doses of RVF vaccine.

| | | Post vaccination HI antibody status | | | |
|---|---|---|---|---|---|
| Number of Doses | Pre-vaccination HI antibody status | >4 fold rise | <4 fold rise | No change | Decrease |
| 3 | Negative 21 | 19 | 1 | 1 | - |
| | Positive 2 | 1 | - | - | 1<br>320-160 |
| 2 | Negative 1 | - | 1 | - | - |
| | Positive - | - | - | - | - |
| 1 | Negative 4 | - | - | - | - |
| | Positive - | - | 3 | 1 | - |
| Total | 28 | 20 | 5 | 2 | 1 |

**Table 5:** Side effects following vaccination with RVF vaccine

| Symptom | Number affected | Number vaccinated | Percentage |
|---|---|---|---|
| Body weakness | 1 | 39 | 2.6 |
| Mild fever | 2 | 39 | 5.1 |
| Body pain | 2 | 39 | 5.1 |
| Localised swelling at site of injection | 1 | 39 | 2.6 |

drop-off at the highest dose level used. According to [18], when inactivated vaccines are used, repeated inoculations of antigen are required to obtain a similar stimulation (as one dose of a live vaccine will produce) with two or three injections given at short intervals followed by a booster injection several months later. Though the antibody level may reach high titres after two or three inoculations, inactivated vacine antibodies have a tendency to drop more rapidly than that of live vaccines. The injection, after the long interval, boosts the production of antibody to high and long lasting levels [18]. The higher antibody titre developed post vaccination by those who were revaccinated three years later is attributed to the presence of memory cells in the immune system. On secondary challenge by the same antigen, the memory cells go into the production of antibodies to that antigen (anamnestic response). The HI test recorded a high percentage (93.1%) of significant seroconversion than the PRN test (86.9%). This is due to the specificity of the PRN test over the HI test. Previous infections with related viruses or exposure to the virus in the laboratory could also have contributed to a higher HI antibody titre. [19] advised that until more is understood about the biology of RVF virus, the HI test can be used only as a crude indication of virus presence, the PRNT being a preferrable index of immunity. In this study, one paired sera showed higher PRNT titre than HI titre. [19] also arrived at a similar result. He observed that a few sera had higher PRN antibody titre than Hi antibodies. This was attributed to the fact that neutralising and HI antigenic determinants of RVF virus may de distinct and the human immune response to them may be under independent control.. Due to the short duration of the antibody induced, it is necessary that a live vaccine be produced which will bring about a long lasting immune response. This is because live vaccine produce naturally by replication, the antigen required for adequate stimulation of cell mediated immunity and antibody response. Another set back of the inactivated vaccine is the possibility that the formalin used might destroy the infectivity as well as a fraction of the antigenicity. This was first observed with formalin inactivated measle virus vaccine. However, the advantage of inactivated vaccine over live vaccine is the negligible percentage of cases of vaccine reverting to infectious form. This is common with live vaccines and is seen causing abortions in animals. The

report of side effects by some vaccines could be attributed to the response of immunocompetent cells to destroy foreign materials. Kark, J.O. *et al.* [16] also recorded side effects following vaccination which include local reactions (e.g erythema, swelling and tenderness or pain at the site of injection) in a group of Israeli high risk individuals in response to vaccination.

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**References:**

1. Davis, BD, Dulbbecco, R, Eisen, NH and Ginsberg, SH. Microbiology, Third Edition. Harper and Row.1980; 1355.
2. Daubney, R, Hudson, JR and Garnham, PC. Enzootic hepatitis or Rift Valley Fever. An undescribed virus disease of sheep, cattle and man from East Africa. J. Path. Bact. 1931; 34: 545-579.
3. Alexander, RA. Rift Valley Fever in the Union. J.S. Afr. Vet. Med. Assoc. 1951; 22: 105-111
4. Shope, RE, Peters, CJ and Davis, FG. The spread of Rift Valley Fever and approaches to it's control. Bull. W.H.O. 1982; 60(3): 299-304
5. Meegan, JM. The Rift Valley Fever epizootic in Egypt 1977-1978. 1. Description of the epizootic and virological studies. Trans. R. Soc. Trop. Med. Hyg. 1979; 73: 618.
6. Ferguson, W.. Identification of Rift Valley Fever in Nigeria. Bull. Epizoot. Dis. Afr. 1959; 7: 317-318
7. Findlay, G.M. Rift Valley Fever or enzootic hepatitis. Trans. R.Soc. Trop. Med. Hyg 1931; 25: 229-262
8. Sabin, AB and Blumberg, RW. Human infection with Rift Valley Fever virus and immunity, twelve years after a single attack. Proc. Soc. Exp. Biol. Med. 1947; 64(4): 385-389.
9. Van Velden, DJJ, Meyer JD, Olivier, J, Gear, JHS and Mclntosh, B Rift Valley fever affecting humans in South Africa. A clinicopathological study. S. Afr. Med. J. 1977; 51: 867-871.
10. Laughlin, LW, Meegan, JM, Stansbaugh, LJ, Morens, D.M and Watten, RH. Epidemic Rift Valley Fever in Egypt: Observations of the spectrum of human illness. Trans. R.Soc. Trop. Med. Hyg. 1979; 73: 630- 633.
11. Fegbami, AH, Tomori, O and Kemp, GE. A survey of Nigeria domestic and wild animals for serum neutralising antibody to indigenous Rift Valley Fever virus. Nig. Vet. J.1973; 2(2): 45-48.
12. Tomori, O. Rift Valley Fever virus infection in man in Nigeria. J.Med. Vir. 1980; 5:343-350.
13. Lennett, EH, and Schmidt, NJ eds. Diagnostic procedures for viral and rickettsial infections. 4th Edition. Amer. Publ. Healt. Assoc. Inc. New York. 1969.
14. Clarke, DH and Casals. Techniques for HA and HA/I test with arthropod-borne viruses. Amer. J. Trop. Med. Hyg. 1958; 7: 561-573
15. Arthropod-borne virus information exchange, Virology Department, University College Hospital, Ibadan, Nigeria. 1977.
16. Kark JO, Aynor, Y.,Mordechai, YB, Ron-Kuper, N, Pelag, BA and Peters., CJ. A serological survey of Rift Valley Fever antibodies in Northern Sinai. Trans. R. Soc. Trop. Med. Hyg. 1982; 76(4): 427-429.
17. Personal Communication
18. Metselaar, D. and Simpson, DIH. Practical virology for medical students and practitioners in tropical countries. Oxford University Press. New York 1982; 496
19. Eddy, GA, Peters, CJ, Meadors, G and Cole, FE. (1981). Rift Valley Fever vaccine for humans. Contrb. Epidemiol. Biostst. 1981; 3:124-141