

SEROLOGICAL STUDIES FOLLOWING VACCINATION WITH ATTENUATED RIFT VALLEY FEVER (RVF) VACCINE IN EGYPT

ELIAN, K. A., WASSEL, M. S., MOHAMED, GIHAN K. M. and El-Debegy, AIDA T.

* Agriculture Research Center, Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

SUMMARY

A total of 1190 serum samples were collected from different species of animals including cattle, buffaloes and sheep for a period of 9 months post vaccination with attenuated RVF vaccine. A serum neutralization test (SNT) was conducted using microtitre plates, BHK cells, the RVF neutralizing antibodies reached 91.9% in sheep, 75.6% in buffaloes and 75.3% in cattle. The result of SNT was confirmed by Indirect Immunofluorescent Assay (IFA) and gave the same result obtained with SNT. The protective level of RVF antibodies (1:40 or higher) obtained from this study lasted for a period of 9 months post vaccination with attenuated RVF vaccine.

INTRODUCTION

Rift Valley Fever (RVF) is an epidemic disease affecting mainly sheep, cattle and other animals as well as human being, causing high economic losses in farm animals due to high mortality rate among young animals (100%) and abortion of pregnant ones (Easterday, 1965).

RVF was introduced to Egypt through importation of infected ruminants or camels from the Sudan (Sellers et al. 1982). Inactivated RVF vaccines were used to protect different farm animals against RVF infection (Abdel Ghaffar et al., 1981, El Nimr et al., 1981 and Marcoss, 1992). However, these vaccines were expensive and of short period of protection. Smithburns

(1949) succeeded to produce an attenuated RVF vaccine which can protect the non pregnant animals for a period of several months. Weiss (1957), in an interesting study vaccinated both ewes and their lambs with an attenuated RVF vaccine with good antibody response without abortion, but signs of encephalitis in their lambs were noticed. The recurrence of RVF disease in Egypt in mild form of infection in 1993 (Gabery et al., 1994) encouraged the General Organization for Veterinary Services to import and use the live attenuated vaccine of Smithburn neurotropic mice adapted strain to protect different farm animals during 1993 - 1994. This vaccine has the advantages of being, not expensive, could be used during epidemics and the duration of protection is long.

The aim of this survey is to study the immune status of different farm animals following the application of imported live attenuated RVF vaccine in Egyptian farms.

MATERIAL AND METHODS

Virus:

Rift Valley Fever virus used in this work was designated as ZHMC₂₁ (Taha, 1982).

Serum:

A total of 1190 samples were obtained from cattle, buffaloes and sheep from different

governorates.

Cell Culture:

Monolayer BHK cell cultures were grown and maintained as described by (El-Karamany, 1981).

Methods:

Serum Neutralization Test (SNT):

Serum neutralization test using tissue culture according to Walker (1975) and titre was calculated according to Reed and Muench (1933).

Indirect Fluorescent antibodies Technique (IFA):

IFA was done by using 96 well tissue culture microplates containing BHK cells infected with 100 TCID₅₀ / ml of RVF virus after 24 hours the infected cells were fixed with absolute Methanoi for at least 20 minutes, washing twice with PBS PH 7.2, addition of serum samples of 2 fold dilution, incubation at 37C for 45 minutes, then

washed with PBS PH 7.2 addition of specific antisera conjugated with FITC, incubated at 37C for 45 minutes and then washed with PBS PH 7.2 then the plate read inverted with fluorescent microscope. This method was modified after Welch and Twiehou (1973) and Patricia et al. (1994).

RESULTS

The results of SN which are shown in Table (4) indicate that the protective level of RVF antibodies reached 90.90 % in sheep, 77.7 % in cattle and 77.6 % in buffaloes, for the whole period of 9 months, where it reached 83 % in sheep, 73.9 % in buffaloes and 60.60 in cattle after 3 months post vaccination as shown in Table (1).

The highest RVF neutralizing antibodies were after 6 months vaccination with attenuated RVF vaccine reached 97.2 % in sheep, 93.6 in buffaloes and 91.0% in cattle as shown in Table (2).

Table (1) : Distribution of RVF neutralizing antibodies among cattle, buffaloes and sheep after 3 months post vaccination with attenuated RVF vaccine.

| Animals | No. of serum | Positive serum 1:40 or higher | | Titre | | | | | | | |
|-----------|--------------|-------------------------------|-------|-------|------|------|------|-------|------|-------|-----|
| | | | | 1:40 | | 1:80 | | 1:160 | | 1:320 | |
| | No. | No. | % | No. | % | No. | % | No. | % | No. | % |
| Cattle | 94 | 57 | 60.60 | 14 | 15 | 19 | 20.2 | 20 | 21 | 4 | 4.4 |
| Buffaloes | 80 | 59 | 73.90 | 10 | 12.5 | 26 | 32.6 | 20 | 25 | 3 | 3.8 |
| Sheep | 53 | 44 | 83.00 | 6 | 11.4 | 15 | 28.3 | 18 | 33.9 | 5 | 9.4 |

Table (2) : Distribution of RVF neutralizing antibodies among cattle, buffaloes and sheep after 6 months post vaccination with attenuated RVF vaccine.

| Animals | No. of serum | Positive serum 1:40 or higher | | Titre | | | | | | | |
|-----------|--------------|-------------------------------|-------|-------|-----|------|------|-------|------|-------|------|
| | | | | 1:40 | | 1:80 | | 1:160 | | 1:320 | |
| | No. | No. | % | No. | % | No. | % | No. | % | No. | % |
| Cattle | 160 | 147 | 91.90 | 5 | 3.1 | 20 | 12.6 | 32 | 20 | 90 | 56.3 |
| Buffaloes | 47 | 44 | 93.60 | 1 | 2.1 | 4 | 8.5 | 11 | 23.4 | 25 | 53.2 |
| Sheep | 36 | 35 | 97.20 | 1 | 2.7 | 5 | 13.9 | 8 | 22.3 | 21 | 58.4 |

Table (3) : Distribution of RVF neutralizing antibodies among cattle, buffaloes and sheep after 9 months post vaccination with attenuated RVF vaccine.

| Animals | No. of serum | Positive serum 1:40 or higher | | Titre | | | | | | | |
|-----------|--------------|-------------------------------|-------|-------|-----|------|------|-------|------|-------|------|
| | | | | 1:40 | | 1:80 | | 1:160 | | 1:320 | |
| | No. | No. | % | No. | % | No. | % | No. | % | No. | % |
| Cattle | 292 | 220 | 75.30 | 4 | 1.4 | 22 | 7.5 | 58 | 19.9 | 136 | 46.6 |
| Buffaloes | 230 | 174 | 75.60 | 6 | 2.6 | 22 | 9.6 | 40 | 17.5 | 106 | 46.0 |
| Sheep | 198 | 182 | 91.90 | 4 | 2.0 | 29 | 14.7 | 41 | 20.7 | 108 | 54.5 |

Table (4) : Protective level of RVF antibodies among cattle, buffaloes and sheep during 9 months post vaccination with attenuated RVF vaccine using SNT and IFA.

| Animals | No. of serum | Positive serum 1:40 or higher using SNT | | Positive serum 1:40 or higher using IFA | |
|-----------|--------------|---|-------|---|-------|
| | | No. | % | No. | % |
| Cattle | 546 | 424 | 77.70 | 424 | 77.70 |
| Buffaloes | 357 | 277 | 77.60 | 277 | 77.60 |
| Sheep | 287 | 261 | 90.90 | 261 | 90.90 |

SNT = Serum Neutralization Test.

IFA = Indirect Fluorescent Antibody Assay.

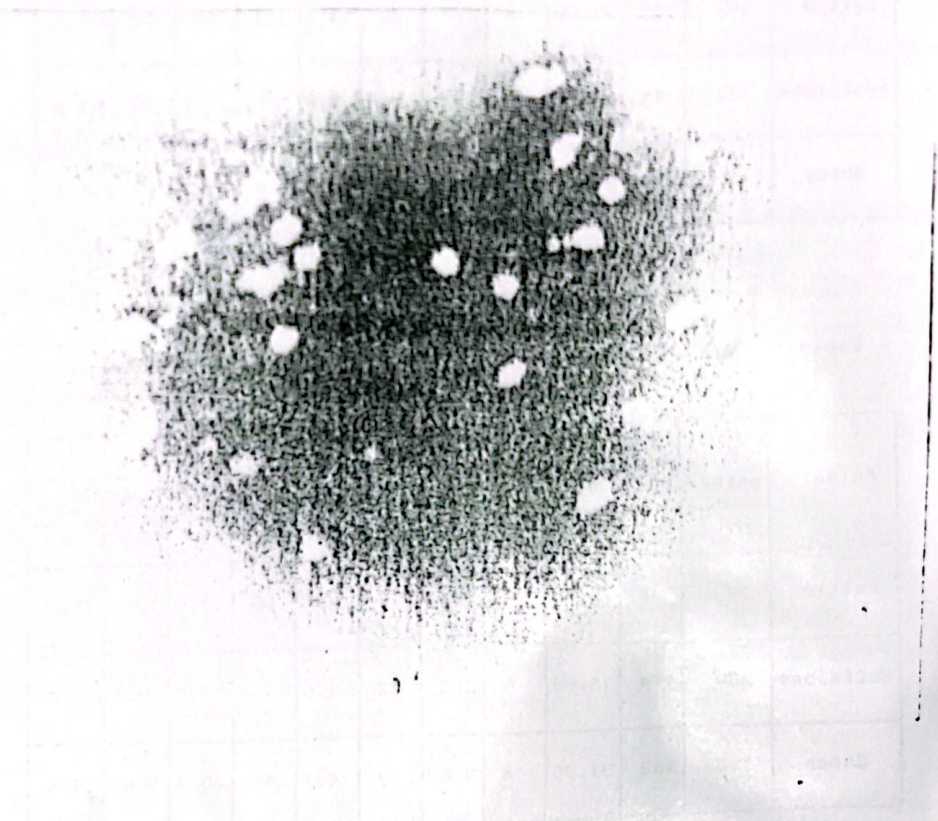


Fig. (1) : BHK cells infected with RVFV (100 TCID₅₀ / ml) react with antibodies against RVF that indirectly react with conjugated antispecies (green fluorescence).

After 9 months post vaccination with attenuated RVF vaccine, the RVF neutralizing antibodies

reached 91.9% in sheep, 75.6% in buffaloes and 75.3% in cattle as shown in Table (3).

The highest titre of SN was 1:320 in sheep, cattle and buffaloes after 6 months post vaccination with attenuated RVF vaccine as shown in Table (2).

The results of SNT was emphasized by IFA and gave the same result as shown in Table (4) and Photo (1).

DISCUSSION

From the previous result of SNT, we could say that the high protective level 1:320 obtained after 6 months post vaccination with attenuated RVF vaccine and were still high in a good protective level > 1:40 in all species of vaccinated animals till 9 months post vaccination. These results were in accordance with protocol of WHO/B5/1392 (1966,1973) (Where the protective level of antibodies following vaccination with attenuated RVF vaccine should be > 1:40, also these results agreed with those obtained by Taha et al. (1994), Morrill et al. (1991), Baskerville et al. (1992) and Hassan (1994). The protective level of neutralizing RVF antibodies was higher in sheep than in cattle and buffaloes. It might be due to species susceptibility.

The protective level of neutralizing RVF antibodies as indicating by SN and emphasized by IFA came in harmony with those of Morrill et al. (1991) and Hassan (1994).

It can be concluded that the protective titre of attenuated RVF vaccine lasted for a period of 9 months which is better than that of inactivated RVF vaccine which protects for a period of 4

months (El Nimr, 1980 and Eman, 1990). Moreover, the attenuated RVF vaccine is not expensive and could be used in the epidemic areas.

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