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**PRELIMINARY STUDIES ON FREEZE-DRIED INACTIVATED RIFT VALLEY
FEVER (RVF)
VACCINES IN EGYPT (With 4 Tables and One
Figure)**

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ف

دراسات أولية على لقاح الريفت فالي المثبط والمجف

ن

محمد واصل ، محمد طه ، الهام الأبياري ، زين محمد ، أحمد ياسي

ا

ح

تم استخدام ٣ خروف لمعرفة قوة رد الفعل المناعي الناتجة عن التحصين بلق حمي الوادي المتصدع المجفف باضافة اللبن المنزوع الدسم الذي تم اختياره من بين اللقاحات

ي

الأخري المجففة والمضاف اليها (الجيلاتين أو الكروز مع اللاكتو البيومين) بناء على نتيجة اختبار القوة العيارية في الفئران السويسرية . تم قياس رد الفعل المناعي عن طريق

ي

ف

اجراء الاختبارات السيولوجية المختلفة (اختبار التعادل - المكمل المثبت والترسيب الآجار). أظهرت النتائج أن رد الفعل المناعي والحماية من العدوي بفيروس حمي الوادي المتصدع عالية في الأغنام .

SUMMARY -

A total of 36 of susceptible sheep used to detect the immune response of alum gel Rift vally fever vaccine (RVF) and the lyophilized skimmed milk RVF vaccine which was selected from other lyophilized ones (gelatin RVF vaccine and sucrose-lact. albumin RVF vaccine) according to their potency test (EDC) in swiss-mice. The immune response was measured by serum neutralization test (SNT), complement

fixation test (CET) and the agar gel precipitation (AGP) test. The alum gel RVF vaccine and the lyophilized skimmed milk vaccine produced a high immunogenic response and good protection from infection with RVF virus in sheep.

INTRODUCTION

Many research centers in Africa succeeded to prepare RVF vaccines to protect animals most of which have advantages and disadvantages. SMITHBURNS (1949) succeeded **ed to produce an attenuated RVF vaccine which can protect the non pregnant animal** for a period of several months. WEISS (1957) in an interesting study vaccinated both

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ewes and their lambs with an attenuated RVF vaccine, **with good antibody response** without abortion but signs of encephalitis in their lambs occurred. In Egypt immediately after the appearance of the disease, in 1977 (IMAM et al., 1978), the Egyptian authorities succeeded in preparing a safe and potent inactivated Alum gel RVF vaccine EL NIMR (1980), TAHA (1982) and EL-KARAMNY (1981) **produced a, potent inactivated RVF vaccine using gelatin as stabilizer. This vaccine was issued and delivered to Egyptian veterinary organization for the vaccination of animals in the government farms up till now. This vaccine may be used immediately or be backed and stored.** The keeping quality of alum gel RVF vaccine is only safe and potent at 4°C for 4 months (IMAN, 1990).

The present work is designated to produce and test several lyophilized safe and potent RVF vaccine with different preservations to determine the immune response to such vaccines.

MATERIAL and METHODS

1 - Virus :

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

Vaccines

a. RVF alum; gel inactivated vaccine prepared according to TAHA (1982).

Freeze-dried inactivated RVF vaccine contain 0.5% gelatin as stabilizer according to (EL-KARAMANY, 1981) . Freeze-dried inactivated RVF vaccine

(EL-NIMR, 1980) contain 10% sucrose and 5% Lact. albumin, equal

amount of each to the stock virus (SOAD, 1986). d. Freez-dried inactivated RVF vaccine (TAHA, 1982) contained 10% skimmed

milk equal amount to the stock virus as described by (SOAD, 1986). Cell culture : Monolayer BHK and CER cell culture were grown and maintained as described by (EL-KARAMANY, 1982).

Animal : – 36 susceptible sheep of 1-2 years age were used. – Swiss albino mice were used for vaccine evaluation and safety.

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Vaccine evaluation and control : The inactivated RVF vaccines were tested for the sterility, safety and potency according to EL-NIMR (1980).

Sero-conversion :

+

test (SNT)

using tissue culture system

according to

Serum neutralization WALKER (1975).

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Complement fixation test (SNT) using tissue culture system according to WALKER (1975). Complement fixation test (CFT) according to (ABDEL GHAFAR et al., 1981). Agar gel precipitation test (AGPT) according to AYOUB and ALLAM (1981).

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Experimental design :

a. A total of 12 sheep were used. Each was vaccinated with RVF alum gel vaccine using 1 ml subcutaneously (S/C) . b. A total of 12 sheep were used, each was vaccinated with inactivated freez-dried RVF skimmed milk vaccine that gave the best EDC (0.0018) from the other lyophilized RVF vaccine using 1 ml S/C after dissolving

it in P.B.S. C. A total of 12 sheep were considered as non-vaccinated control. d. Challenge was performed 42 days post vaccination against RVF using a dose of 10 TCID/ml.

Sera samples were collected weekly after vaccination and daily after challenge with RVF virus collected sera were kept at -70°C until used.

RESULTS

The results of safety test of all tested vaccines as shown in table (1). The was virus free. The ED₅₀ of the alum. gel RVF vaccine was 0.0013, as good as that of the lyophilized skimmed milk RVF vaccine (0.0018) but the ED₅₀ of the lyophilized gelatin RVF-vaccine (0.019) and that of the lyophilized sucrose-lactalbumen RVF vaccine (0.02) is considered to be accepted.

As shown in table (2) and figure (1) the immune response of sheep to the alum. gel RVF vaccine the NI reached (2.8) on the 6th week post vaccination while that of the lyophilized skimmed milk RVF vaccine NI reached (2.5) on the 6th week post vaccination. Non of the immunized sheep developed febrile reaction or detectable viraemia or other symptoms of infection during 10 days following challenge with 10 TCID /ml of RVF virus.

The results of CFT of vaccinated sheep sera (in both group vaccinated either with alum gel RVF or lyophilized skimmed milk RVF vaccine) revealed that CF antibodies were detected 14 days post-vaccination and the titer reached 1/16 on the 42nd day post-vaccination as in table (3).

Table (4) revealed that the results of AGPT were agreed with those obtained from SNT and CFT. the antibodies appeared from the 2nd week till the 6th week post-vaccination with both kind of RVF vaccines.

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DISCUSSION

The EDs of all tested vaccines of RVF gave ED. less than 0.02, while gave a good immunity and protection since the achieved NI reach from (2.5-2.8) in the 6th week post-vaccination, that means definite protection from infection (EASTERDAY et al., 1982; RANDAL et al., 1964; WALKER, 1975; EL-NIMER, 1980 and TAHA, 1982). The results of CF were agreed with those obtained by ABDEL GHAFAR et al. (1981) and TAHA (1982) who indicated the presence of specific CF antibodies against

RVF vaccine in sera of vaccinated sheep reached 1/16. The results of AGPT were agreed with those obtained by AYOUB and ALLAM (1981) and IMAN (1990) who indicated the presence of a precipitating lines from the 2nd to 6th weeks post-vaccination with RVF vaccine.

From the above all mentioned we can say that, the proper inactivation with proper concentration are essential for the successful production of RVF vaccine. As no big difference between the immune response in sheep, of both alum gel and lyophilized skimmed milk RVF vaccine, lyophilization of the vaccine using 10% skimmed milk as a virus stabilizer and as an adjuvant beside inactivation with 0.2% formalin was successful.

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0 -- 0 Alum gel RVF vaccine *-.-* Lyophilized
skimed milk RVF vaccine
challenge

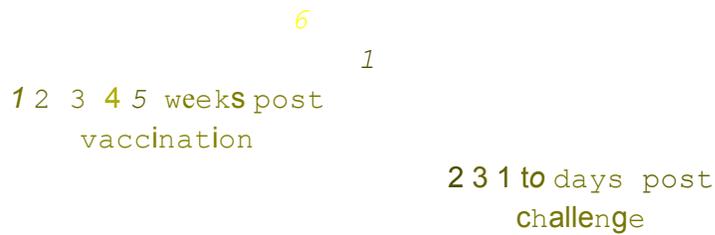


Figure 1: NI of sheep vaccinated with alum gel RVF vaccine and lyophilized skimmed milk RVF vaccine.

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Table (1) : Potency of RVF prepared vaccines as measured by ED50/0.1 ml.

Kind of inactivated RVF Vaccine.

Tioration before inactivation 10⁹10 TCID₅₀/ml

Percent of formalin as inactivator

ED50 in mice

Gelatin-RVF. Vac. Sucrose-Lac-alb.-RVF

8.5

0.2%

0.019

Vac.

8.5

0.2%

0.02

Skimmed milk RYF-Vac. Alum.ge!. RVF. Vac.

8.5 8.5

0.2% 0.2%

0.0018 0.0013

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Table (2) : Mean of RVF neutralizing indices in vaccinated sheep as well as after challenge)

Animal group

Mean Ni titres of sheep

Weeks post vaccinations

2 3 4 5

6

Days after challenge 1 2

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1.2

1.4

1.9

2.5

2.8

2.6

2.2

2.3

2.9

- Sheep vaccinated

with alum.gel. RVF.vac. 0.9 – Sheep vaccinated with Lyophilized skimed-

- 0.6

0.0

3.3

1.1

1.1

1.2 1.2

1.6 1.6

2.2 2.2

2.5 2.

2.4

2.1

2.2

2.7

3.2

milk-vaccine

Table (3): Complement fixation on post vaccination collected sheep sera.

Kind of vaccines

Animal

Mean of CF titre days post vaccination

14

28

Alum gel RVF. vacc.

12 sheep

12 sheep

Lyoph.skimed Milk RVF vaccine.

Table 4: Results of AGPT on sera from sheep at various intervals

post-vaccination

with RVF vaccines

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vaccines

Animal

AGPT at AGPT week, post vacc.

Alam gel RVF. vacc. Lyoph. skimed Milk RVF. vaccine

12 sheep 12 sheep

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+ = a precipitin line.