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EFFECT OF DIFFERENT ADJUVANT ON THE QUALITY OF ANTI-RIFT VALLEY FEVER HYPERIMMUNE SERUM

(With 4 Tables)

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تأثير المحسنات المختلفة على كفاءة السيرم المناعى لفيروس حمى الرفت فالى

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محـــاولات لإنـــتاج ســـيرم عالى المناعة ضد فيروس حمى الرفت فالى فى الأرانب البلدى باســـتخدام أنواع مختلفة من المحسنات (saponin, IMS3013, IMS 1113 and Fruend's) وكانـــت النســـبة المســـتخدمة من هذه المحسنات هى ٠,٥% بالنسبة للصابونين. أما بالنسبة للمحســـنات الأخـــرى فكانـــت النســـب متساوية (١:١). وتم حقن كل محسن من المحسنات المختلفة فى مجموعة من الأرانب (ثلاث جرعات من الفيروس المثبط بالإضافة إلى المحسن بيـــن كل جرعة أسبوع ثم الجرعة الرابعة من الفيروس الضارى فقط تم حقنها بعد اسبوع. وأثبتـــت النتائج باستخدام التجارب السيرولوجية المختلفة: التعادل المصلى، الاليزا، الترسيب فـــى الأجـــار أن اســـتخدام IMS 3013 فـــى إنتاج مصل عالى المناعة يعتبر أجود أنواع المحسنات المختلفة حيث أن القوة العيارية للأجسام المناعية الموجودة فى هذا المصل وصلت إلى ١٢٨٠ فى الفترة من ١٠-١٥ يوم واستمرت حتى اليوم ٢١ بعد نهاية الحقن. أما بالنسبة لباقى الأنواع فكانت أعلى نسبة من الأجسام المناعية فى اليوم العاشر بعد أخر حقن للأرانب.

SUMMARY

Trials for production of hyperimmune sera against Rift Valley Fever (RVF) virus in balady rabbits were done by using different types of adjuvants (saponin, IMS 3013, IMS 1113 and Freund's adjuvants). The concentration of saponin was 0.5% added to the vaccine while the other adjuvants were added in equal amounts to the vaccine. Each group of rabbits received four injections subcutaneously with one week interval, the first 3 injections were the mixture of inactivated virus and adjuvant but the last one was the virulent virus only. The level of antibody of the prepared hyperimmune sera was evaluated and determined by 3 different serological tests (serum neutralization test "SNT", ELISA and agar gel

precipitation test). They revealed that the best of choice adjuvant was IMS3013 which gave high antibody titre at the 10^{th}-15^{th} day post last injection and persist till the 21^{st} day, the other types of adjuvants gave high level of antibody at 10^{th} day post injection but less in titre than that obtained by using IMS3013.

Key words: *Adjuvant, Rift Valley Fever, hyperimmune serum*

INTRODUCTION

Rift Valley fever virus is a member of genus Phlebovirus in the family Bunyaviridae (Connie, 1996). RVF is one of the most important arthropod born viral diseases in Africa, primary affecting domestic animals with occasional involvement of man (Peters and Meegan, 1981).

The disease is most sever in sheep, cattle and goats causing high mortalities and abortion in pregnant animals. That disease runs in a rapid course with short incubation period (Swanepoel and Coetzer, 1994 and OIE, 1996).

RVF was recorded for the first time in Egypt during 1977-1978 affecting sheep, cattle and human (WHO, 1978). The second epidemic of RVF was documented in 1993 in animals as well as in human being in Aswan governorate (El-Gabery *et al.,* 1994). Another epidemic of the disease occurred in 1997 (Abdel Rahim *et al.,* 1999). Rapid diagnostic tools are needed to detect RVF in both human and animals to control the disease. Therefore, the aim of the present work was planned to prepare hyperimmune sera against RVF virus in rabbits using different adjuvants such as saponin, immunosol oil IMS3013, IMS1113 and Freund's adjuvants and to detect the best one that gives high level of antibody titre to be used for rapid detection of RVF disease for emergency diagnosis.

MATERIALS and METHODS

Material:

Animals:

1. Adult mice:

21-28 days old Swiss albino mice susceptible to RVF were used for virus titration and toxicity test for different adjuvants used in preparation of hyperimmune serum.

2. Baby mice:

3-5 days old Swiss albino mice susceptible to RVF were used for measuring the toxicity of used adjuvants.

3. Rabbits:

25 balady rabbits of 4-6 months old and weighed 1.5-2 kgm were used for preparation of polyclonal RVF hyperimmune sera.

Virus:

Rift valley fever (RVF) virus strain diagnosed as ZH501 and had a titre of $10^{7.5}$ $TCID_{50}$/ml. It was kindly supplied from RVF Dept., Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

RVF vaccine:

Tissue culture binary inactivated Rift valley fever (RVF) vaccine was prepared according to Eman (1995).

Agar gel precipitation antigen:

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

RVF antigen:

Lyophilized RVF cell lysate antigen was used for indirect ELISA and prepared according to Elian and Botros (1997).

Adjuvants:

1. Saponin:

It was obtained as a powder from KC Hlight LTD, England and prepared as 10% solution in double distilled water. It was kept overnight at 4°C then filtered through Seitz (EKS) filter (Marcoss *et al.,* 1998).

2. Montanide IMS oil adjuvants:

They were obtained from Seppic, Paris, France. Each type of oil IMS 3013 and IMS 1113 was initially mixed at equal volume with tris NaCl buffer (v/v), pH (7.6). Then each one was added in equal volume to the inactivated RVF virus and low stear mixing at 250-300 rpm for 5 minutes was required (Barnett *et al.,* 1998).

3. Complete and incomplete Freund's adjuvants:

They were supplied by BCG Dept., Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

Toxicity test:

Adult mice as well as baby mice were used for the safety of adjuvants used in preparation of hyperimmune sera. Intracerebral inoculation of baby mice and intraperitoneal inoculation of adult mice with different types of adjuvants and then observed for 10 days post inoculation.

Addition of adjuvants:

- Saponin:

It was added with 0.5 % to RVF binary inactivated vaccine following Marcoss *et al.* (1998).

- Complete and incomplete Freund's adjuvants:

Complete and incomplete Freund's adjuvants were added in equal amount to RVF antigen according to Green and Manson (1990) and Zeidan *et al.* (2000).

Preparation of polyclonal RVF hyperimmune sera in rabbits (Experimental Design):

25 balady rabbits were classified into 5 groups, each group contains 5 rabbits as follows:

Group I: It was inoculated S/C with 1ml. Mixture of saponin and inactivated RVF vaccine 0.5ml in each site of the thigh of rabbit. The inoculation was repeated weekly for further 3 weeks except the last injection was 1ml of virulent RVF virus has a titre of 10^7 $TCID_{50}$/ml.

Group II: It was inoculated S/C with 1ml of the mixture of inactivated RVF virus and IMS 3013 in equal volumes and complete the injection in the same manner as groups (I).

Group III: It was inoculated S/C using IMS 1113 as in group (II).

Group IV: It was inoculated S/C with 1ml inactivated RVF virus and incomplete Freund's adjuvant and the other two injections by using complete Freund's adjuvant instead of incomplete Freund's adjuvant and the last injection with RVF virus alone.

Group V: Control non-inoculated group.

All groups of rabbits were observed for any signs of illness and bled from the marginal ear vein at the 5^{th}, 10^{th}, 15^{th}, 21^{st} days post of last injection, the sera were separated for measuring the level of antibody titre by using:

1. Serum neutralization test:

It was performed according to Swanepel *et al.* (1986).

2. Indirect enzyme linked immunosorbent assay (ELISA):

It was applied according to Voller *et al.* (1976).

3. Agar gel precipitation test:

It was applied according to Eman (1990).

RESULTS and DISCUSSION

Rift valley fever is an acute arthropod born viral disease affect many species of animal and human, causing high mortalities among lamb and abortion in pregnant animals (Swanepoel and Coetzer, 1984).

Reappearance of RVF epidemic in Egypt during 1981 and 1993 demonstrated that this disease could extend its geographical boundries and cause serious human and animal disease as detected for the first time in Saudi Arabia (Fagbo, 2002).

The aim of this study is to prepare standard hyperimmune sera to be used as diagnostic tools for early detection of RVF using different kinds of adjuvants and compare between them.

Result of toxicity due to different adjuvants in baby and adult mice in shown in Table (1). It revealed that the non-toxic percentage of saponin which can be added to the inactivated vaccine suspension is 0.5% and this result was in agreement with Marcoss *et al.* (1998) while the two percentages of IMS 3013 and IMS 1113 which added to the inactivated virus was 50% which gave no deaths in mice and this result agree with Ali and Roshdy (2004). Also, the incomplete and complete Freund's adjuvants gave non-toxic results when used in equal amount with the inactivated RVF virus. These results agree with Zeidan *et al.* (2000).

All types of hyperimmune sera prepared using different adjuvants were tested to determine the level of antibody titre represented in Table (2) showed that the highest level of antibody titre reached at the 10-15 days post last injection in all kinds of adjuvants used, but the highest titre obtained by using IMS 3013 and IMS 1113 oil adjuvants (1280) and continue for 21 days post the last injection for IMS 3013 and these results are in agreement with Barnett *et al.* (1998) who said that IMS 3013 is a new generation of oil adjuvant termed (immunosol) which form micro-emulsion it includes new immunostimulants, listed as grass substance which elicit both humoral and cell-mediated immune response.

According to the results of ELISA, Table (3) indicated that the highest level of antibody titres represented at 10-15 days post injection (1280) for IMS 3013 while IMS 1113 the highest titre was at 10 days only. Regarding to saponin and Freund's adjuvant, they gave a titre of 640 at 10 days post immunization. These results were in agreement with Taradi (2003) who found that the highest antibody titre reached at 10^{th} day post last injection.

Table (4) showed the results of agar gel precipitation test of prepared hyperimmune sera which confirm the same results showed by SNT and ELISA as cited by Samy *et al.* (2001) they found that Freund's adjuvant used for preparation of hyperimmune sera give the highest titre at 10 days post injection.

The conclusion of the present work is that the application of oil adjuvant IMS 3013 was the best of choice for increasing and persistence of high level of antibody.

Table 1: Toxicity of different types of adjuvants used in preparation of hyperimmune sera against RVF

Adjuvant	Percentage (%)	Baby mice (I/C)	Adult mice (I/P)
Saponin	1	7/7 *	* 8/10
	0.5	0/7	0/10
IMS 3013	50	0/7	0/10
	25	0/7	0/10
IMS 1113	50	0/7	0/10
	25	0/7	0/10
Complete and incomplete Freund's	50	0/7	0/10
Control	-	0/7	0/10

* Number of dead mice / Number of tested mice.

Table 2: Mean serum neutralizing antibody titre of hyperimmune sera against RVF virus using different adjuvants

Groups of rabbits	Treatment (adjuvant)	Before Immunization	Days post immunization			
			5	10	15	21
Group (I)	Saponin	0	80	640	640	320
Group (II)	IMS 3013	0	640	1280	1280	1280
Group (III)	IMS 1113	10	160	1280	1280	640
Group (IV)	Fruend's	0	160	640	640	320
Group (V)	Control	10	10	10	10	10

Table 3: Results of ELISA test of hyperimmune sera against RVF virus using different adjuvants

Groups of rabbits	Treatment (adjuvant)	Before Immunization	Days post immunization			
			5	10	15	21
Group (I)	Saponin	0	20	640	640	80
Group (II)	IMS 3013	0	320	1280	1280	640
Group (III)	IMS 1113	0	160	1280	640	640
Group (IV)	Fruend's	0	320	640	160	160
Group (V)	Control	0	10	10	10	10

Table 4: Mean agar gel precipitating antibody titre of hyperimmune serum against RVF virus using different adjuvants

Groups of rabbits	Treatment (adjuvant)	Before Immunization	Days post immunization			
			5	10	15	21
Group (I)	Saponin	0	20	320	320	80
Group (II)	IMS 3013	0	160	640	640	640
Group (III)	IMS 1113	0	160	640	640	320
Group (IV)	Fruend's	0	80	320	640	80
Group (V)	Control	0	0	0	0	0

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