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COMPARATIVE STUDIES OF IMMUNE RESPONSE BETWEEN BALADY AND IMPORTED SHEEP VACCINATED WITH INACTIVATED RIFT VALLEY FEVER VACCINE

(With 3 Tables)

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(Received at 10/6/2006)

دراسات مقارنة للإستجابة المناعية بين الأغنام البلدى والمستوردة المحصنة بلقاح حمى الوادى المتصدع المثبط

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فى هذه الدراسة تم استخدام إثنى عشر من الأغنام، ستة منهم من الأغنام البلدية، وستة أخرى من الأغنام المارينو المستوردة، تم حقن أربعة أغنام من كل نوع بلقاح حمى الوادى المتصدع المثبط وترك أربعة بدون حقن كضوابط للتجربة وفى البداية تم إستخدام عدد إثنين من الحملان البلدى وإثنين من الحملان المارينو لإجراء إختبارالسلامة للقاح المستخدم فى التجربة حيث تبين سلامة اللقاح وفاعليته. تم أخذ عينات سيرم من الأغنام لمدة ستة أشهروثم الكشف على الأجسام المناعية فى عينات السيرم بإستخدام الأختبارات السيرولوجية (التعادل المصلى والإليزا) والتي أظهرت أن الأجسام المناعية فى الأغنام البلدى تظهر مبكرا وكان المستوى المناعى بدأ يظهر من الأسبوع الثانى عن الأغنام المارينو وأن المستوى المناعى بعد سته أشهر من التحصين متقارب فى النوعين مما يعكس لنا أن الأغنام البلدى كانت أسرع فى الأستجابة المناعية للقاح وأكثر حساسية عند تقييم اللقاح.

SUMMARY

Inclusive studies were conducted to estimate the humoral immune response of balady and marino sheep post vaccination with inactivated Rift Valley Fever (RVF) vaccine, firstly the safety test in lambs and the potency test in mice were done. The specific developed RVF antibodies were monitored for six monthes by both serum neutralization test (SNT) and enzyme linked immunosorbant assay (ELISA). The studies indicated that the balady sheep is earlier to respond than marino sheep. Hence it could be used efficiently for evaluation of the produced inactivated RVF vaccine.

Key words: *Rift valley fever, vaccination, immune response*

INTRODUCTION

Rift Valley Fever (RVF) is an acute arthropod borne viral disease occurred in Egypt for the first time in 1977 and 1978 causes sever economic losses with dramatic levels of humans infection (Meegan and Moussa, 1979). RVF was also reoccurred in Egypt in 1994 (Zaghawa and Elian, 1995) and in 1997 (Abd-El Rahim *et al.*, 1999). RVF formalin inactivated vaccine is currently used in Egypt since1978 for vaccination of sheep and cattle (El-Nimr, 1980). Then the development of the prepared vaccine inactivated by binary etheleinimine (BEI) was safe and potent for vaccination of sheep than formalin (Eman, 1995). Evaluation (sterlity, safety and potency) of the prepared vaccine were done (O I E, 2005). Continous vaccination programs are required using inactivated RVF vaccine one ml s/c for sheep (Taha *et al.*, 2001).

The aim of this work is to compare between immune response of balady and imported sheep after vaccination with the inactivated RVF vaccine

MATERIALS and METHODS

1- Animal:

A- Balady sheep: six susceptible sheep 8 month old; 4 sheep were injected with RVF inactivated vaccine and other 2 sheep were kept as a control.

B- Marino sheep: six, susceptible sheep 8 month old imported from Australia; 4 sheep were injected with RVF inactivated vaccine and other 2 sheep were kept as a control.

C- Lambs : two susceptible balady and two marino lambs with their dam (5- 10 days old) were used in safety test of RVF vaccine and observed 10 days according to Gihan (1990) and OIE (2005).

D – Mice:

1- Two groups of seven suckling Swiss albino mice (3 – 5 days) are used in safety test; one group is inoculated intracerebraly (I/C) with 0.03 ml of inactivated vaccine without alum gel and second group is kept as control (Gihan, 1990 and OIE, 2005).

2- Adult mice (21 -30 days old) were used in potency test was carried out as described by (Randal and Harison, 1964 and OIE, 2005), the effective dose fifty (ED_{50} /ml) was calculated according to the method described by Reed and Muench, (1938).

2- Preparation and evaluation of inactivated RVF vaccine:

RVF virus strain (ZH 501) was kindly supplied by RVF vaccine Department, Serum and vaccine Res. Inst. Abbassia, Cairo. The obtained virus was inactivated by Binary Etheleinimine (Eman ,1995) and the evaluation, (sterility, safety and potency tests) were carried out according to the protocol illustrated by OIE (2005).

3- RVF antigen and antisera were supplied from RVF vaccine Dept. Serum and Vaccine Research Institute Abbassia Cairo.

4- Experimental design:

Group 1: 4 Balady susceptible sheep vaccinated S/C with 1 ml 10^7 tissue culture infective dose fifty (10^7 $TCID_{50}$/ml) inactivated RVF virus vaccine .

Group 2: 4 Marino susceptible sheep vaccinated S/C with 1 ml (10^7 $TCID_{50}$/ml) inactivated RVF virus vaccine

Group 3: 2 Balady and other 2 Marino sheep were unvaccinated control.

5- Sero-converstion tests :

A- Serum neutralization test (SNT) was applied according to the methods described by Walker (1975). using BHK tissue culture cells.

B- Enzyme linked immunosorbant assay (ELISA) was carried out as prescribed by Voller *et al.* (1976).

RESULTS

Table 1: Evaluation of the prepared inactivated RVF vaccine.

Vaccine	sterility	Safety			Potency ED_{50}
		Suckling mice	Balady lamb	Marino Lamb	
Binary inactivated RVF vaccine	Sterile	0/10*	0/2*	0/2*	0.003ml

* = numbers of deaths or showing symptoms / total number

Table 2: Mean results of Neutralizing Antibody index (NI) in sera of balady and marino sheep vaccinated with inactivated Rift Valley Fever vaccine:

Animal Groups		No. of Animals	Means NI							
			Weeks post. Vaccination							
			Pre Vaccination	2	4	8	12	16	20	24
Gp. 1 Balady		4	0.5	1.5	1.7	2.0	2.7	2.2	2.0	1.7
Gp. 2 Marino		4	0.3	1.2	1.5	2.1	2.5	2.0	1.8	1.5
Gp. 3 Control	Ba.	2	0.7	0.5	0.4	0.8	0.3	0.4	0.5	0.4
	Ma.	2	0.5	0.3	0.5	0.4	0.5	0.4	0.3	0.5

Gp. =group Ba.=balady sheep
Ma.= marino sheep NI.= Neutralizing indices

Table 3: Mean of ELISA readings (Optical density) in sera of balady and marino sheep vaccinated with inactivated Rift Valley FeverVaccine.

Animal groups		No. of animals	Weeks post. Vaccination							
			Pre vaccine	2	4	8	12	16	20	24
Gp. 1 Balady		4	0.008	0.094	0.113	0.129	0.189	0.169	0.158	0.145
Gp. 2 Marino		4	0.004	0.086	0.099	0.101	0.163	0.141	0.107	0.139
Gp. 3 control	Bal.	2	0.006	0.007	0.002	0.008	0.007	0.008	0.008	0.003
	Mar.	2	0.003	0.009	0.009	0.06	0.003	0.008	0.005	0.002

Cut off = 0.07

When injected the sterile, safe and potent inactivated RVF vaccine from the data illustrated in Table (1) by estimating the ED_{50} of the prepared inactivated RVF vaccine revealed a figure 0.003 ml which is acceptable and valid for use as cited by Randal and Harison (1964) and Taha *et al.* (1984). They reported that the accepted figure of ED_{50} was not more than 0.02/ml. The sera of vaccinated balady and marino sheep were evaluated by using SNT and ELISA techniques to follow up the humoral immunity for six monthes after vaccination.

Regarding (Table 2) the neutralizing indices of the vaccinated groups, it was found different reading as 1.5 and 1.2 (NI) in 2nd week after vaccination in Balady and Marino sheep respectively. The estimation of neutralizing antibodies as immunoresponse level in vaccinated sheep with inactivated sterile, safe and potent RVF vaccine appeared from the2nd week post vaccination (Gihan, 1990). The rise of NI readings is continuously elevated over the protective level (1.5) which estimated by Pini *et al.* (1973).

The neutralizing indices (NIcs) were in a protective level (1.7 and 1.5) till the end of six month post vaccination. Concerning the Elisa technique, the obtained results (Table 3) elucidated that there were slightly difference between the native breed sheep and marino, and the O.D. peaked at 12th week post vaccination. The O.D value was calculated according to the method described by Edward (1985). The obtained values of O.D. appeared to be more sensitive than SNT between the two types of sheep and these findings were agreed with (Niklason *et al.*, 1984 and Meegan *et al.*, 1987).

In conclusion,the native breed sheep may have a protective level earlier than marino that a mean the Balady sheep can response rapidly for defense against the disease after vaccination than the Marino sheep. This study also revealed that Balady sheep more better than Marino sheep in evaluation of RVF vaccine.

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