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SOME STUDIES ON SHEEP VACCINATED WITH SMITHBURN ATTENUATED RIFT VALLEY FEVER VACCINE

HASSAN, K. E. Z., KHAIRAT A. ELIAN AND M. M. TAHA

Veterinary Serum and Vaccine Research Institute, Agricultural Research Centre, Ministry of Agriculture, Dokki, Giza, Egypt.

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Abstract

This study was performed on 9 adult sheep divided into 3 groups, one group included nonpregnant vaccinated sheep, second group was pregnant vaccinated ewes and the third group was nonpregnant non-vaccinated kept as control. Some of the vaccinated animals showed a slight elevation of body temperature. Two pregnant ewes delivered normal lambs, while the 3rd ewe was aborted one month after vaccination. RVF virus was isolated from different organs of the aborted foetus. No significant changes were found in total protein, Serum Glutamic Oxaloacetic Transaminase (GOT) and Serum Pyrovic Transaminase (GPT) than values as compared with non-vaccinated control animals. However, there was significant decrease in albumin value and significant increase in globulin value than in control animals. The neutralizing antibodies were still detectable at protective level till 36 months post-vaccination and the peak was reached at the 3rd month post-vaccination. The immunoglobulins as detected by Enzyme Linked Immunosorbent Assay (ELISA) were recorded as optical density reading and the results were correlated with those obtained by Serum Neutralization Test (SNT).

INTRODUCTION

Rift Valley Fever (RVF) is a zoonotic, acute, viral disease that primarily affects domestic animals, and occasionally causes disease in humans. RVF may cause severe disease in both animals and humans with high morbidity and mortality, and exacting substantial economic loss of livestock. RVF is most commonly associated with mosquito-borne epidemics during years of heavy rainfall. An epizootic of RVF is generally observed during years, typically occurs in 5 to 20- year cycles, in which heavy rainfall and localized flooding occur. The excessive rainfall allows mosquito eggs, usually of the genus Aedes, to hatch. The mosquito eggs are naturally infected with the RVF virus, and the hatched mosquitoes transfer the causative virus to livestock on which they feed. Once the livestock is infected, other species of mosquitoes can become infected from the animals and can spread the disease. In addition, it is possible that the virus can be transmitted by other biting insects (WHO, 1998). The incubation period of RVF varies

from two to six days. There, then follows an influenza-like illness, with sudden onset of fever. The first indication of development of an epidemic is frequently the abortion of sheep. The simultaneous occurrence of numerous cases of abortion and disease in ruminants, together with disease of humans, following heavy and prolonged rainfall, is characteristic of RVF (WHO, 1998). Clinical disease has been observed in sheep, goats, cattle, domesticated Asian buffaloes, camels and humans (WHO, 1998).

RVF can be prevented by a sustained programme of animal vaccination. Both inactivated and live attenuated vaccines have been developed for veterinary use. The live vaccine requires only one dose and produced long-lived immunity (WHO / OMS, 1998). Smithburn live attenuated vaccine, widely used in Africa, is highly immunogenic and presents a suitable candidate vaccine for the control of RVF (Botros *et al.*, 1995).

In this work, we followed-up the serologic immune response of adult sheep vaccinated with live attenuated RVF vaccine until the protective level of immune response has vanished. Moreover, determination of some serum biochemical constituents was carried out at different time intervals post-vaccination.

MATERIALS AND METHODS

Animals

Nine adult sheep (3 of them were pregnant), were used for follow-up the effect of vaccination (immune response) and other parameters.

Biological Reagents

RVF antigen

Lyophilized RVF cell lysate for IgG detection by ELISA was used (Elian and Botros, 1997).

Conjugate

Antisheep horse-reddish peroxidase. Purchased from Sigma Company.

Biochemical Kits

Total protein kit, Albumin kit, GOT and GPT kit, all were obtained from Biocon Company (Germany).

RVF vaccine

Smithburn neurotropic strain at its 102nd mouse brain passage, batch No. 10 was produced by RVF department at the Vet. Serum and Vacc. Research Institute, Abbassia, Cairo, and evaluated by Control lab. at Vet. Serum and Vacc. Research Institute.

RVF virus

ZH 501, isolated from a human patient in Zagazig province (WHO, 1998), was used for SNT.

METHODS

Detection of total protein: according to Josephson and Gyllensward (1975).

Detection of albumin: according to Webster (1974).

Detection of GOT and GPT: according to Reitman and Frankel (1957).

Virus isolation: Isolation of the vaccinal virus from vaccinated aborted ewe was carried out (Walker *et al.* 1970).

Seroconversion

1. Serum neutralization test (SNT): used to detect specific neutralizing antibody against RVF virus according to the method of Walker *et al.* (1970).

2. Enzyme Linked Immunosorbent Assay (ELISA): used to detect IgG against RVF virus according to the method described by Voller *et al.* (1976).

Experimental Design

The animals were divided into 3 groups:

- Group one: included 3 non-pregnant vaccinated sheep.
- Group two: included 3 pregnant vaccinated ewes.
- Group three: included 3 non-pregnant non-vaccinated sheep, kept as control.

All groups were clinically observed till the end of experiment, and serum samples were collected for detection of total protein, albumin, GOT, GPT and specific antibody to RVF virus.

RESULTS

Four vaccinated animals showed slight elevation of body temperature (about 0.5°C) for one day (on 4th day) post-vaccination. Two pregnant ewes delivered normal lambs, while, the 3rd ewe was aborted one month after vaccination (at the 3rd month of pregnancy). RVF virus was isolated from different organs (liver, spleen and brain) of the aborted foetus, but intestine and kidney were free. No significant changes were found in total protein, GOT and GPT as compared with values of non-vaccinated control animals. There was significant decrease in albumin values and significant increase in globulin value when compared with control animals (Tables 1 &2).

The neutralizing antibodies were detected at protective level (NI 1.7 $\log_{10}$ $TCID_{50}$) till 36 months; the peak was reached at the 3rd month post-vaccination (NI 3.5 $\log_{10}$ $TCID_{50}$), while, at 39th month post-vaccination, the level decreased to be non-protective (NI 0.9 $\log_{10}$ $TCID_{50}$) as shown in Table 3.

The immunoglobulins (IgG) were detected by ELISA and recorded as optical density readings, and the results correlated well with those obtained by SNT as shown in Table 4.

Table 1. Mean of total proteins, albumin and globulin in sera of sheep vaccinated with attenuated RVF vaccine as compared with controls.

Groups of animals	No. of animals	Unit / ml sera																											
		Weeks post- vaccination																											
		Zero			1st			2nd			3rd			4th			5th			6th			7th			8th		
		TP	A	G	TP	A	G	TP	A	G	TP	A	G	TP	A	G	TP	A	G	TP	A	G	TP	A	G	TP	A	G
G1	3	6.8	2.9	3.9	6.8	2.4	4.4	6.9	2.2	4.7	7.0	2.0	5.0	7.6	1.8	5.8	7.2	1.7	5.5	6.9	1.6	5.3	6.9	1.7	5.2	6.8	1.6	5.2
G2	3	6.7	3.0	3.9	7.0	2.3	4.4	7.1	2.2	4.8	7.0	2.1	4.9	7.7	1.9	5.8	7.3	1.7	5.6	7.0	1.6	5.4	6.9	1.6	5.3	6.9	1.6	5.3
G3	3	6.6	2.6	4.0	6.9	2.5	4.4	6.7	2.5	4.2	6.8	2.4	4.4	6.8	2.5	4.3	6.5	2.4	4.1	6.2	2.3	4.3	6.5	2.4	4.1	6.2	1.9	4.3

TP = total protein, A= albumin, and G = globulin.

Table 2. Mean of GOT and GPT values of sheep sera vaccinated with RVF vaccines as compared with control sheep.

Group	No. of animals	unit / ml sera													
		Weeks post vaccination													
		Zero		1st		2nd		3rd		4th		5th		6th	
		GOT	GPT	GOT	GPT	GOT	GPT	GOT	GPT	GOT	GPT	GOT	GPT	GOT	GPT
G1	3	54.6	13.5	53.5	14	56.3	13.5	55.1	11.3	56.5	11	53.8	12.66	54	12
G2	3	54	11.8	53.3	12	54	12.8	55	13.1	55.8	13.5	53.5	12.3	52	12.4
G3	3	53.6	12.3	54	12.4	54.6	12.3	54.3	12	54.3	12.5	54.6	12.1	53	11.8

Table 3. Mean of neutralizing indices in sera of sheep vaccinated with attenuated RVF vaccine as well as control sheep.

Group of animals	No. of animals	Mean of neutralizing indices																
		Time post-vaccination																
		Day	Week	Months														
		Zero	1st	1st	2nd	3rd	6th	9th	12th	15th	18th	21st	24th	27th	30th	33rd	36th	39th
G1	3	0.5	1.7	2.2	2.7	3.5	2.8	2.3	2.0	2.0	1.9	1.8	1.8	1.7	1.7	1.7	1.7	0.9
G2	3	0.6	1.7	2.1	2.6	3.3	3.0	2.4	2.1	2.0	1.9	1.9	1.8	1.8	1.7	1.7	1.6	0.8
G3	3	0.5	0.5	0.6	0.6	0.6	0.5	0.6	0.5	0.6	0.5	0.6	0.6	0.5	0.6	0.6	0.6	0.5

Table 4. Mean of ELISA optical density reading in sera of sheep vaccinated with attenuated vaccine as compared with control sheep.

Group of animals	No. of animals	Mean of ELISA optical density																
		Time post-vaccination																
		Day	Week	Months														
		Zero	1st	1st	2nd	3rd	6th	9th	12th	15th	18th	21st	24th	27th	30th	33rd	36th	39th
G1	3	0.045	0.110	0.16	0.26	0.39	0.29	0.175	0.145	0.14	0.13	0.125	0.121	0.119	0.119	0.11	0.11	0.086
G2	3	0.042	0.110	0.155	0.25	0.37	0.31	0.19	0.15	0.14	0.13	0.13	0.121	0.121	0.119	0.119	0.110	0.08
G3	3	0.040	0.054	0.05	0.05	0.05	0.05	0.06	0.05	0.045	0.05	0.04	0.06	0.05	0.045	0.06	0.045	0.050

Cut off = 0.09 reading on plate reader at 492 nm wavelength.

DISCUSSION

In the present studies, elevation of body temperature of vaccinated sheep (0.5°C) on the 4th day post-vaccination, agreed with Gihan *et al.* (1996) and Hassan (1998) who reported that sheep vaccinated with live attenuated RVF vaccine showed elevated body temperature post-vaccination as post-vaccination reaction. The abortion occurred in one ewe at the 3rd month of pregnancy, which agreed with Ibrahim (1996), but disagreed with Gihan *et al.* (1996) and Hassan (1998), probably due to the less number of animals used in this study. Non-significant changes were found in the total protein, GOT and GPT as compared with control values which agreed with El-Sawalhy *et al.* (1997) and Mouaz *et al.* (1998) but disagreed with Gihan *et al.* (1993) who reported an increase in total serum protein post-vaccination with attenuated RVF vaccine. Albumin values showed significant decrease than that of controls, while, globulin value showed significant increase than that of controls that agreed with Hassan (1998), but disagreed with Mouaz *et al.* (1998) who reported no significant changes. Duration of immunity, which, was detected by SNT and ELISA tests, revealed that the immunity in protective serological limit lasted for 36 months post-vaccination that agreed with WHO / OMS (1998). This result led to the conclusion that approximately one dose of live attenuated RVF vaccine is sufficient for protection of sheep against the disease during their life.

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بعض الدراسات على الأغنام المحصنة بلقاح حمى الوادي المتصدع المستضعف عترة سميث برن

كريم الدين زكى حسن ، خيرات عبد المجيد عليان ، محمد محمود طه

معهد بحوث الأمصال واللقاحات بالعباسية، مركز البحوث الزراعية، وزارة الزراعة، الدقى، جيزة، مصر.

تمت هذه الدراسة على عدد ٩ أغنام قسمت إلى ثلاث مجموعات. المجموعة الأولى حصنت باللقاح الحي المستضعف والمجموعة الثانية كانت عشار وحصنت بنفس اللقاح والمجموعة الثالثة تركت كضابط للتجربة. أظهرت النتائج إرتفاعاً طفيفاً في درجة الحرارة لبعض الأغنام المحصنة وحدثت حالة إجهاض واحدة من مجموعة الأغنام العشار المحصنة وتم عزل الفيروس من أعضاء الجنين. بالنسبة للتحاليل البيوكيميائية التى أجريت على المصل لم يحدث تغيير في قيمة البروتين الكلى للمجموعات الثلاث وكذلك قيم الإنزيمات الدالة على وظائف الكبد ولكن كانت قيمة الألبيومين أقل وقيمة الجلوبيولين أعلى من قيمتها في مجموعة ضابط التجربة. وبالنسبة للإختبارات السيرولوجية أظهرت النتائج إرتفاع مستوى الأجسام المناعية في الحيوانات المحصنة لتصل إلى أعلى مستوى في الشهر الثالث بعد التحصين واستمرت الأجسام المناعية بمستوى كافي لوقاية الأغنام ضد المرض حتى الشهر ٣٦ بعد التحصين.