EFFECT OF ATTENUATED MP12 RIFT VALLEY FEVER VACCINE ON IMMUNE RESPONSE OF SHEEP

SAYED, TARADI A. [1], A. M. IBRAHIM[1], LILY S. SALAMA[1], T. N. MARCOS[1] AND A. K. SOLIMAN[2]

1 Veterinary Serum and Vaccine Research Institute, Ministry of Agriculture, ARC, Dokki, Giza, Egypt
2 NAMRU – Cairo

(Manuscript received 30 March 2011)

Abstract

This work aimed to study the clinical aspects and immune response of pregnant and non-pregnant ewes vaccinated with attenuated MP12 RVF vaccine. Non-pregnant ewes showed slight elevation of body temperature lasted for one day post-vaccination. There were no detectable clinical signs of RVF disease. Pregnant ewes showed no elevation in body temperature, no abortion and delivered healthy lambs (without abnormalities). RVF virus was detected on the 2nd day post-vaccination in 15 out of 18 serum samples using RT- PCR. IgM antibodies were detected on 7 days post-vaccination (DPV) and persisted up to 150 DPV with a titer ranged from 100 – 800. IgG antibodies were detected on 7 DPV reaching the peak on 60 DPV and persisted up to 360 DPV with a titer ranged from 100 – 800. Maternal immunity appeared in newly born lambs after ingestion of colosrum and lasted up to 4 months. It is concluded that MP12 RVF vaccine is safe and potent for vaccination of sheep.

INTRODUCTION

Rift Valley Fever (RVF) is a peracute or acute, febrile mosquito born zoonotic disease caused by a virus of the family *Bunyaviridae*, genus *Phlebovirus*. The disease is characterized by high rate of abortion and neonatal mortality primarily in sheep, goat and cattle (OIE, 2008). Natural occurrences of RVF were first documented in Egypt in 1978, where killed more than 600 people and caused sickness to over 200,000 (Meegan, 1979), and another outbreak in 1993 (MMWR, 1994). An outbreak in Yemen in 2000 was the first documented occurrence outside Africa (MMWR, 2000).

Vaccine development is focusing on protecting both animals and humans. One of the most promising is the live attenuated vaccine candidate MP-12. This vaccine was developed by researchers at the US Army Medical Research Institute of Infectious Diseases (USAMRIID). The vaccine has proved efficacy and safety in sheep and cattle (Caplen, *et. al.,* 1985). One dose of attenuated virus vaccine proved long term immunity, but not better in pregnant animals. The inactivated virus vaccine doesn't have side effect, but multiple doses were given to provide protection (WHO, 2007).

Immunoglobulin – M (IgM) could be detected through out the period from the 7th day up to 21st day post-vaccination, and persisted for a short duration when the live attenuated RVF vaccine was used, but was not detectible in case of inactivated vaccine (Elian and Botros, 1997), while immunoglobulins – G (IgG) lasted for 36 months post-vaccination with live attenuated smithburn vaccine (Hassan, *et al,* 2001).

ELISA was reported as safe, robust, and highly accurate technique used in early diagnosis of infection, disease surveillance and for monitoring of immune response in vaccines (Paweska, *et. al.,* 2005).

This work aimed to:

1. Evaluate the clinical aspects of the mutagenized Rift Valley Fever MP12 vaccine in pregnant and non-pregnant local sheep breed.
2. Assess the post-vaccination immune response (IgG & IgM) and duration of immunity.
3. Assess the safety of the MP12 vaccine (abortoginicity & teratogenicity) during the study.

MATERIALS AND METHODS

Twenty-one Balady sheep (less than 3 years old) were divided into three groups, first one (G1) (14 non-pregnant ewes) and 2nd one (G2) (4 pregnant ewes at 1st trimester) were vaccinated with 10^5 PFU attenuated MP12 vaccine, while, the 3rd one (control) (3 sheep) was kept as non-vaccinated negative control. All groups were examined daily for any clinical signs, and rectal temperatures were recorded till 6 days post-vaccination (DPV). Sheep of pregnant group were observed till parturition and nursing.

Lyophilized RVF-ZH548-MP12 mutagenized vaccine was supplied by NAMRU – 3. This vaccine was developed by workers at the US army Medical Research Institute for Infectious Diseases (USAMRIID) by serially passaging of a human virus isolate (strain ZH548) in human diploid fibroblast cells in the presence of the mutagen 5-fluorouracil (Caplen *et. al.,*1985).

Sheep sera samples were collected from all groups on 0, 2, 7, 14, 28, 60, 120, 150, 210 and 360 DPV. Lamb sera samples were collected before colosrum ingestion and 2, 30, 60, 90 and 120 days after colosrum ingestion.

Determination of immunoglobulin (IgG) and (IgM) against RVFV was carried out using ELISA technique (titer≤1:50 consider positive) according to OIE (1989).

RT – PCR for detection of RVF RNA was done according to Sall *et. al.* (2002) on sera samples from vaccinated sheep on zero and 2nd DPV.

RESULTS AND DISCUSSION

Table 1. Temperature and clinical signs of sheep vaccinated with MP12 RVF vaccine.

		Animal No.	Temperature (^{O}C) results and clinical signs									
			Days post-vaccination									
			zero		1		2		3	4	5	6
			M	E	M	E	M	E	M	M	M	M
G1	Non-pregnant ewes	1	39 -	39.7	39.3	39.7	39.2 +	39.2	39	39.3	38.4	38.2
		2	38.8 -	39.5	39.4	39.3	39.4 +	39.2	39.7	39.5	38.7	38.0
		3	38.9 -	39.5	38.4	39.2	39 -	39.4	39.7	38.4	38.6	38.2
		4	39 -	39.3	39.5	40	40 +	39.8	39.2	38.4	38	38.1
		5	39 -	39.5	39.5	39.5	39 +	39.1	39.9	38.7	38.2	38.7
		6	38.9 -	39.7	41	40.8	39.4+	39.9	39.8	38.6	38.2	38.5
		7	39 -	39.5	39.8	40.5	39.1 -	39.5	39.3	38.7	38.3	38.7
		8	38.9 -	39.6	39.3	39.8	39.9 +	39.3	39.5	38.5	39.0	38.5
		9	38.8 -	39.2	40.2	40.1	39 +	39.2	38.4	38.4	38.3	38.9
		10	38.6 -	39.7	40.3	40.1	39.5+	39.7	38.7	39	38.5	38.0
		11	39 -	39.3	39.8	39.9	39 +	39.7	38.5	38.5	38.4	39.1
		12	39 -	39.5	40.5 D	40.2 D	39.5 D+	39.2	38.4	39.7	39	39.0
		13	38.9 -	39.8	40.2	40.1	39.1 +	39.9	38.4	39.7	38.4	39.0
		14	39 -	39.8	40.9	40.8	39.3 +	39.8	38.7	39.2	38.7	39.0
G2	Pregnant sheep	1	38.6 -	39.8	38.9	39.5	38.8 -	39.7	38.6	39.9	39.0	38.6
		2	39 -	39.7	39.3	39.6	39.3 +	39.7	38.7	39.8	38.7	38.3
		3	38.9 -	39.7	39.6	39.6	39.2 +	39.3	38.5	38.4	38.5	39.1
		4	38.6 -	39	39.5	39.2	38.5 +	39.5	38.4	38.7	38.4	39.0
Non-vaccinated Control		1	38.5 -	38.4	38.6	38.5	38.7	38.4	38.4	38.7	38.6	38.6
		2	38.7 -	38.7	38.4	38.6	38.5	38.4	38.7	38.5	38.4	38.6
		3	38.6 -	38.6	38.4	38.7	38.4	38.6	38.6	38.4	38	38.4

E = evening M = morning D = Diarrhea + = PCR + - = DPV PCR -

Table 2. Results of immunoglobulin (IgM) titer of sheep vaccinated with MP12 RVF vaccine using ELISA technique.

		Animal No.	IgM titer					
			Days post-vaccination					
			0	7	14	28	60	150
G1	Non-pregnant ewes	1	-ve	200	200	200	100	100
		2	-ve	400	400	400	100	100
		3	-ve	100	200	100	100	100
		4	-ve	200	400	200	200	200
		5	-ve	400	400	100	100	100
		6	-ve	100	200	100	100	100
		7	-ve	100	100	100	100	100
		8	-ve	200	400	100	100	100
		9	-ve	400	400	100	100	100
		10	-ve	100	200	100	100	100
		11	-ve	200	200	200	100	100
		12	-ve	400	400	800	800	800
		13	-ve	400	400	100	200	100
		14	-ve	100	100	100	100	100
G2	Pregnant sheep	1	-ve	100	200	200	200	100
		2	-ve	100	200	200	100	100
		3	-ve	100	100	100	100	100
		4	-ve	200	400	200	100	100
Non-vaccinated Control		1	-ve	-ve	-ve	-ve	-ve	-ve
		2	-ve	-ve	-ve	-ve	-ve	-ve
		3	-ve	-ve	-ve	-ve	-ve	-ve

Table 3. Results of immunoglobulin (IgG) titer of sheep vaccinated with MP12 RVF vaccine using ELISA technique.

		Animal No.	IgG titer							
			Days post-vaccination							
			0	7	14	28	60	150	210	360
G1	Non-pregnant ewes	1	-ve	50	100	100	200	200	200	100
		2	-ve	50	100	400	400	200	100	100
		3	-ve	100	400	400	800	800	800	800
		4	-ve	100	400	400	400	400	400	200
		5	-ve	100	200	400	800	400	200	100
		6	-ve	50	100	400	400	200	200	200
		7	-ve	50	100	400	200	200	200	200
		8	-ve	50	100	200	200	200	200	100
		9	-ve	100	200	200	200	200	200	100
		10	-ve	50	400	800	800	800	400	200
		11	-ve	100	200	200	200	400	200	100
		12	-ve	50	100	200	200	200	200	100
		13	-ve	50	100	200	200	200	200	100
		14	-ve	100	200	200	200	400	200	100
G2	Pregnant sheep	1	-ve	50	100	200	400	400	200	100
		2	-ve	50	100	200	200	200	400	200
		3	-ve	50	200	400	400	200	200	200
		4	-ve	100	200	400	400	400	200	100
Non - vaccinated Control		1	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		2	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
		3	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Table 4. The mean titer of immunoglobulins M and G (IgM & IgG) of sheep vaccinated with MP12 RVF vaccine using ELISA technique.

Sheep groups		Mean titer / Days post-vaccination							
		0	7	14	28	60	150	210	360
IgM	G1	-ve	235.7	285	192.8	164.7	157.1	0	0
	G2	-ve	125	225	175	125	100	0	0
IgG	G1	-ve	71.5	192.5	321.4	371.4	342.8	264.2	178.5
	G2	-ve	62.5	150	300	350	300	250	150

Table 5. The titer of IgG of lamb born from sheep vaccinated with MP12 vaccine using Elisa technique.

Lamb No.	Time / days post-colosrum ingestion						
	before	2	30	60	90	120	150
1	-ve	800	800	400	200	100	>50
2	-ve	400	400	200	100	50	>50
3	-ve	400	400	100	100	50	>50
4	-ve	800	800	400	200	50	>50
Mean	-ve	600	600	275	150	62.5	>50

Clinical observations of all sheep groups vaccinated with MP12 RVF vaccine were shown in Table 1. The results revealed that one sheep out of 18 ones on 1st and 2nd DPV had diarrhea. Six sheep In (G1) recorded febrile response (40 – 40.9 ^{O}C), which lasted for 2 days and returned to normal body temperature. These results

agreed with Morrill *et. al.* (1991*)* who recorded that pyrexia was observed in some lambs vaccinated with MP12 RVF vaccine for short duration post-vaccination.

Pregnant ewes group (G2) showed no elevation of body temperature and delivered healthy lambs after 100 days post-vaccination. Results of RT- PCR for RVF virus detection in vaccinated sheep groups (G1 &G2) on zero day were negative, while, on 2nd day post-vaccination, it was found that 15 out of 18 serum samples were positive. This finding explains the short duration of vireamia (2 days).

Pregnant ewes (G2) showed no abortion and delivered lambs without abnormalities and remained healthy up to 4 months of age. These results agreed with Baskervillee *et. al.* (1992) who reported that abortion is not common sequel after vaccination of pregnant ewes with live attenuated Smithburn RVF vaccine.

The immune response following vaccination of sheep with MP12 vaccine was carried out using ELISA technique.

Table 2 showed that IgM antibodies were detected on 7 DPV in vaccinated sheep reaching the peak level on 14 DPV and persisted up to 150 DPV in all animals with a titer which ranged from 100 – 800 ELISA. This result agreed with Hassan *et. al.* (2009) who reported that IgM titer reached its peak after 14 days post-vaccination (DPV) of calves vaccinated with MP12 vaccine, while this titer decreased till 28 DPV.

Tables 3 & 4 demonstrated that IgG antibodies were detected on 7DPV, reaching the peak on 60 DPV and persisted up to 360 DPV in all animals, with a titer which ranged from 100 – 800 ELISA. These results agreed with Hassan *et. al.* (2001).

Table 5 declared immunity of lambs born to MP12 vaccinated pregnant ewes which were negative at birth before ingestion of colosrum, then, increased to 600 ELISA titer after colosrum ingestion till 30 days, then, decreased reaching 62.5 at 120 days. This result agreed with Morril *et. al.* (1987) who found that lambs born to MP12 vaccinated pregnant ewes had antibody level less than 1:10 antibody titer at birth, and increased to 1: 80 after ingestion of colosrum.

The obtained results indicated that single dose of MP12 RVF vaccine is immunogenic and safe for non-pregnant and pregnant ewes. The duration of immunity was recorded up to one year post-vaccination. Lambs protective antibodies were detected after colosrum ingestion, and persisted up to three months proving the efficiency of the vaccine to protect lambs against RVF virus infection.

REFERENCES

1. Baskerville, A., K. A. Hubbard and J. R. Stephanson. 1992. Comparison of the pathogenicity for pregnant sheep of Rift Valley Fever V and live attenuated vaccine.Res. Vet. Sci., 52 (3) : 307 – 311.
2. Caplen, H., C. J. Peters and D. H. Bishop. 1985. Mutagen-directed attenuation of Rift Valley fever virus as a method for vaccine development. J. Gen. Virol., 66:2271-7.
3. Elian, K. A. and B. Botros. 1997. Production and evaluation of RVF diagnostic antigens by ELISA technique.3rd Arab Vet. Med. Cong. March 22-26,1997 Cairo.
4. Hassan, K.Z, K. A. Elian and M. M. Taha. 2001. Some studies on sheep vaccinated with smithburn attenuated Rift Valley Fever vaccine. Egypt .J.Agr.Res.,79 (3) :1141-1149.
5. Hassan, K. E. Z., A. M Ibrahim, A. S.Taradi, K.Elian, A. M. H. Azab, H. El-Mohamady, and A. K.Soliman. 2009 .The effect of Rift Valley Fever vaccines on the release of interferon and immunoglbulin - M in vaccinated cattle Alex. J. Vet. Sci. 28 (1) 110-2047.
6. Meegan, JM. 1979. The Rift Valley fever epizootic in Egypt 1977-78. 1. Description of the epizootic and virological studies. Trans. R. Soc. Trop. Med. Hyg.,73: 618-623.
7. Morb IMortal Wkly (MMWR). 1994. International Notes: Rift Valley Fever - Egypt, 1993. MMWR Morb Mortal Wkly Rep.,43:693,699-700.
8. Morb Mortal Wkly MMWR. 2000.Outbreak of Rift Valley Fever -- Yemen August--October 2000. MMWR Morb Mortal Wkly Rep.,49:1065-1066.
9. Morril, JC., G. B., Jenning, H. Caplen, M. Turell, A. J. Johnson and C. J. Peters. 1987. pathogenicity and immunogenicity of a mutagen-attenuated Rift valley Fever immunogen in pregnant ewes. Am. J. Vet. Res., 78(7):1042 -1047.
10. Morrill, J. C., L. Carpenter, D. Taylor, H. H. Ramsburg, J. Quance and C. J. Peters. 1991. Further evaluation of a mutagen-attenuated Rift Valley fever vaccine in sheep. Vaccine, 9(1):35-41.
11. Office of International Epizootics (OIE). 2008. Manual of Diagnostic tests and vaccines for terrestrial Animals.6th Ed, Vol. 1: 323 – 333.
12. Office of International Epizootics (OIE). 1989. Manual of recommended diagnostic techniques and requirements for biological products of lists A and B diseases.International Committee Vol. 2 P. 1/8 - 8/8.

13. Paweska,J.T., F.J. Burt and R. Swanepoel. 2005. Validation of IgG-sandwich and IgM-capture ELISA for the detection of antibody to Rift Valley fever virus in human.J. Virol. Methods, 124 (1-2) : 173-81.
14. Sall, A. A., E. A. Macondo, O. K. Sène, M. Diagne, R. Sylla, M. Mondo, L. Girault, L. Marrama, A. Spiegel, M. Diallo, M. Bouloy and C. Mathiot. 2002. Use of Reverse Transcriptase PCR in Early Diagnosis of Rift Valley Fever. American Society for Microbiology, Clinical and Diagnostic Laboratory Immunology, p. 713-715, Vol. 9, No. 3.
15. World Health Organization (WHO). 2007. Rift Valley Fever WHO Media centre, Fact sheet N°207.

تأثير لقاح حمى الوادي المتصدع المستضعف MP12 علي الأغنام

تراضى عبد الفتاح سيد[1] ، ألفونس مينا إبراهيم[1] ، للي صبحي سلامة سعد[1] ، تيمور مرقص نصيف[1] ، عاطف كامل سليمان[2]

1 معهد بحوث الأمصال واللقاحات البيطرية – مركز البحوث الزراعية – وزارة الزراعة – الدقى – الجيزة

2 NAMRU – القاهرة

تم دراسة الأعراض المرضية والإستجابة المناعية للنعاج العشار وغير العشار المحصنة بلقاح حمى الوادي المتصدع المستضعف MP12 .

أظهرت النعاج الغير عشار إرتفاعاً طفيفاً في درجات الحرارة التي استمرت لمدة يومين بعد التحصين ولم تظهر أية أعراض لمرض حمى الوادي المتصدع، بينما لم تظهر النعاج العشار أي إرتفاع في درجات الحرارة أو إجهاض وتم ولادة حملان طبيعية.

باستخدام تقنية RT-PCR تم الحصول علي 15 حالة إيجابية لفيروس حمى الوادي المتصدع من 18 عينة مصل في اليوم الثاني بعد التحصين. وباجراء إختبار الإليزا ظهرت الأجسام المناعية IgM في اليوم السابع واستمرت حتى 150 يوماً بقوة عيارية من 100 – 800 ، بينما ظهرت الأجسام المناعية IgG في اليوم السابع بعد التحصين واستمرت حتى 360 يوماً ، أما بالنسبة للحملان فقد ظهرت الأجسام المناعية بعد رضاعتها للسرسوب بقوة عيارية 600 . واستمرت المناعية الأمية لمدة 4 شهور.

من النتائج السابقة يتضح أن لقاح حمى الوادي المتصدع المستضعف MP12 آمن وفعال لتحصين الأغنام.