EVALUATION OF THE IMMUNITY FOLLOWING VACCINATION WITH ATTENUATED RIFT VALLEY FEVER VACCINE IN CATTLE

By

Nirmeen G. Shafiek*; Arwa H. El nagger* and Wassel, M.S.*
**Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, Cairo, Egypt.*

SUMMARY

When attenuated Rift Valley Fever (RVF) vaccine was evaluated in cattle induces poor antibody response where the antibody level reached (>40) for 3 months then decreased after 4th month with serum neutralization test and 910-2210 with ELISA test for 3 months.

INTRODUCTION

Rift valley fever virus, an arthropod borne virus principally of cattle and sheep , the disease is characterized by a febrile illness, liver necrosis and in severe cases death frequently associated with hemorrhagic symptoms (Findlay,1932 and Gerdes, 2002)

RVF was first recognized in Kenya 1931 as described by Daubney et al. (1931) ,further extensive outbreaks of the disease were extended to southern Africa and several other countries of Africa (Weiss, 1957). Pregnant cows were aborted and few only died, where serological evidence showed that not all cattle became infected (Swanepoel et al., 1979).

Rift valley fever (RVF) was first reported in Egypt in a periodic severe epizootics among animals and were frequently accompanied by epidemics in humans (Meegan,1979; EL-Akkad,1981). A major epidemic of RVF was introduced to Egypt through imported infected ruminants or camels from Sudan (Sellers et al., 1982) causing infections, abortion and deaths in sheep, cattle, goats, water buffaloes, camel and human (Swanepoel, 1976 and Swanepoel et al., 1979).

Several prophylactic and control measures are recommended, however, immunization of susceptible animals is considered the most effective mechanism to control the disease (Meslin, 1993). Inactivated RVF vaccine was used to protect

different farm animals (Abdel Ghaffar et al., 1981 and El Nimer et al., 1981 and Elian et al., 1997). However, those vaccines and have a shelf life of only 6 months. Smithburn (1949) prepared an attenuated RVF vaccine which can protect the non pregnant animals for a period of several months, although it may induce abortions, fetal anomalies and neonatal death if inoculated into pregnant animals (Easterday, 1965) and was poorly immunogenic in cattle (Swanepoel, 1981)

The aim of this work is to illustrate the immune status of cattle following the application of Smithburn live attenuated RVF vaccine in cattle.

MATERIALS AND METHODS

A- Materials

1-Virus

Rift valley fever virus used in this work was ZH501 supplied by Rift Valley Fever Dep., VSVRI, Abbassia, Cairo, Egypt.

2-RVF antigen and antisera:

-The purified reference RVF antigen and purified bovine anti-RVF immunoglobulin were kindly supplied from RVF Dept., Vet. serum and vaccine research institute (VSVRI).

-Rabbit antibovine IgG conjugated with peroxidase (sigma immunochemical company No. A7414), used in ELISA

-Rabbit antibovine IgG conjugated with flourescein isothiocyanate (FITC) (sigma immunochemical company No. F7882) used to study identity.

-CellCulture:

Monolayer BHK cell culture was grown and maintained as described by (El-Nimer, 1980).

-RVF vaccines:

The attenuated Smithburn RVF vaccine was kindly administered by RVF department, VSVRI, Egypt.

Experimental design : seven susceptible cross breed calves (Frisian and local) about 1 year of age were kept under observation for 7 days before vaccination. General clinical examination was carried out and serum samples were collected for detection of RVF antibodies. Animals were randomly divided into 2 groups Group (1): five calves were vaccinated with 2 ml contain $2X10^{4.5}$ $TCID_{50}$/ml S/C (O.I.E, 1996). To study the duration of

immunity, serum samples were collected post vaccination and SNT, ELISA tests were done.
Group (2): two calves were kept as control negative (non vaccinated).

B. Methods:

1.Titration of RVF virus
RVF virus was titrated using microplate method, according to (Walker,1979)

2.Evaluation of the vaccine
2.1.Purity and sterility test:
the vaccine must be free from bacteria, mycoplasma and fungi contamination.according to the United States Code of Federal Regulations (1987) testing 9 CFR 113.26
2.2.Virus identification:
According to the (WHO, 1983) using direct fluorescent antibodies technique (DFAT) according to (Johnson et al., 1981).
2.3.saftey test:
According to (O.I.E, 1996)
a-Two susceptible hamster were injected with 0.5 ml field dose of vaccine I/P and two were kept as a control and were observed for 14 days.
b- Two susceptible sheep were injected S/C with 25 field dose vaccine, and two sheep injected I/V with 25 field dose of the vaccine, two sheep were kept as a control, and were kept under observation for 21 days
c- Two susceptible cattle 1-2 years old of mixed Balady and Frisian type were inoculated with 10 field dose S/C of the vaccine, one cattle were left as a control, and were observed for 21 days.
2.4.Potency test:
According to (O.I.E, 1996)
Four sheep inoculated with 1 field dose S/C and were bled after 21 days post vaccination and the antibody response were measured by SNT.
2.5.Seroconversion in cattle post vaccination with live attenuated RVF vaccine:
a. Serum Neutralization Test (SNT)
SNT was done to measure the neutralizing antibody titer in sera from vaccinated cattle according to the method of (WHO, 1973).
b-Enzyme Linked Immuno Sorbent Assay (ELISA)
(Nirmeen, 2002, and Voller ,1976).

RESULTS AND DISCUSSION

From the previous results we could say that the titer of the used vaccine was (6.5 log10 MLD_{50}/ml) in mice and (7.5 log10$TCID_{50}$/ml) in BHK cells .The vaccine was safe in hamster ,sheep and cattle which agreed with WHO, 1983 and OIE 1996 protocols, and protective antibody level were >40 in vaccinate sheep for >12 months (Nirmeen, 2002), but when this vaccine experimentally tested for potency and immunogenicity in cattle the antibody levels were (>40 with SNT test and 910-2210 in ELISA test) for 3 months then decreased ,and become poorly immunogenic in vaccinated cattle, this might be because Smithburn strain of attenuated RVF vaccine could not stimulate the immune system of cattle for long duration to produce enough antibodies for protection against RVF infection ,this agrees with (Swanepoel 1981) who said that the attenuated Smithburn vaccine is poorly immunogenic in cattle and (Elian et al., 1996) where they found antibodies against RVF attenuated Smithburn vaccine till the 6^{th} month and in low level for 9 months. Hence, we could say that another strain of attenuated RVF is needed to protect cattle instead of Smithburn vaccine against RVF infection for control measures in cattle specially during outbreaks, (Morrill et al., 1987 and Morrill et al., 1997).

It was concluded that, the duration of immunity is short following vaccination of cattle with attenuated Smithburn vaccine when compared it when used in sheep.

We can say that using attenuated Smithburn RVF vaccine in cattle is of short term of immunity of low immunogenicity which might be due to strain variation of RVF or due to species susceptibility. So the attenuated RVF vaccine (Smithburns) should not be used in vaccination policy in cattle as a whole in pregnant or non-pregnant. So it is important to prepare a new vaccine which give a high immune response in cattle instead of Smithburn to control RVF infection in cattle.

REFERENCES

Abdel Ghaffar, S.; El Nimr, M.M.; Mohsen, A.Y.; El Nakashly, S.; Attia, M. and Emad, N. (1981). Seroconversion of sheep vaccinated with inactivated Rift Valley Fever vaccine. Bull. Del'office Internationale des Epizootic, 39 (11-12):1379-1385.

Briggs, D.J. and Skeels, J.K. (1984). An enzyme linked immunosorbent assay for detecting antibodies to pasteurella multocida in chickens. Avian Dis. 28(1): 208-215.

Daubney, R.; Hudson, J.R. and Graham, P. C. (1931). Rift valley fever or enzootic hepatitis as undescribed diseases of sheep, cattle and man from East Africa. J Path Bact., 34 (1): 545-579.

Easterday, B. C. (1965). Rift Valley Fever. Adv. Vet. Sci., 10 (65): 127.

Elian, K.A.; Gihan, K.M. and Eman, M.S. (1997). Production and keeping quality of the attenuated Rift Valley Fever vaccine. 4th scient. Cong. Egyp. Societ. For cattle diseases. 7-9 Dec. Assiut. Egypt.

Elian, K.A.; Wassel, M.S.; Gihan, K.M. and El-Debegy, A.T. (1996). Serological studies following vaccination with Rift Valley Fever vaccine in Egypt. 4th scient. Cong. Egyp. Societ. For cattle diseases. 3-7 April. Vet. Me. J. Giza 44 (2) 409-417.

El-Akkad, A.M. (1981). Recent epidemiological status of RVF in Egypt from 1975-1980. J. Egyp. Public Health Assoc. LVI (5 and 6): 351-355.

El Nimr, M.M. (1980). Studies on the inactivated vaccine against RVF. pH.D. Thesis (Microbiology) Fac. Vet. Med. Assiut Univ. Egypt.

El Nimr, M. M.; Abdel Ghaffar, S.; Mohsen, A.Y.; EL Debegy, A.; El Danaf, N.; El Nakashly, S.; Mohamed, Z. and Emad Nafie. (1981). Infection of BHK cells with RVFV. Bull. Of Int. Epiz., 93(11-12): 1351-1359.

Fidlay, G.M. (1932). Rift Valley Fever or enzootic hepatitis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **25**, 229-265.

Gerdes, G.H. (2002). Rift Valley Fever. Vet. Cli. North. Am. Food. Anim. Pract. Nov; 18(3): 549-555.

Johnson, K.M.; Elliatt, L.H. and Heyman, D. L. (1981). Preparation of polyvalent viral immunofluorescent intracellular antign and use in human sero-surveys. J. Clin. Micbiol., 14: 527-529.

Meegan, JM.; Hoogstral, H. and Moussa MI. (1979). An epizootic of RVF in Egypt in 1977. Vet. Rec. 105: 124-125.

Meslin, F.X. (1993). Report of the WHO/IZSTe consultation on recent development in RVF (with the participation of FAO and OIE). Civitella del Tronto. 1-23.

Morill,J.C.;Jennings,G.B and Caplen,H.(1987). Pathogenicity and immunogenicity of a mutagen attenuated Rift Valley Fever virus imunogen in pregnant ewes .Am .J.Vet.Res. 1042-1047

Morill,J.C.;Mobus ,C.A.and Peters ,C.J.(1997). Satey of a mutagen attenuated Rift Valley Fever virus vaccine in fetal and neonatal bovids . Am.J.Vet.Res.1110-1114

Nirmeen, G.S.; (2002). Studies on the evaluation of modified living attenuated Rift Valley Fever virus vaccine. PhD thesis, ?Virology, Fac. Of Vet. Med., Cairo Univ.

O.I.E. (1996). Office Internationale Des Epizootics. World Organization for Animal Health. Manual of standards for diagnostics tests and vaccines.

Sellers, R. F.; Peolyley, P.E. and Tucken, M.R. (1982). Rift Valley Fever, Egypt 1977, disease spread by windborne insect vectors. Vet. Rec. 110: 73-77.

Smithburn, K.C. (1949). RVF. The neurotropic adaptation of virus and experimental use of this modified virus as a vaccine. Br. J. Exp. Path., 30: 1-6.
Swanepoel R., (1976). Studies on the epidemiology of Rift Valley Fever. J. of the South Afric. Vet. Med. Assoc. 47: 93-94.
Swanepoel R., (1981). Rift Valley fever in Zimbabwe, proccedings of the 49th General session of the office international de Epizootic, Paris-75-30 May.
Swanepoel R.; Manning, B. and Watt, J.A. (1979). Fatal Rift Valley Fever affecting humans in south Africa; a clinical pathological study. Cent. Afric. J. Medc. 25: 7-8.
United State Code of Federal Regulations (1987). Animal and animal products, 9, 1987. published by the office of the Federal Register National Archieves and Records Administration.
Voller, A. (1976). ELISA for the immunodiagnosis of virus infection in Rose, N.R. Fridman, H. (ed.). Manual of Clinical Microbiol., Washington, D.C. Amer. Soc. Microbiol., 506-512.
Walker, J.S. (1979). Citied after EL Nimr, M.M.H., personal communication.
Weiss, K. E. (1957). Rift valley fever. A review Bull. Epis. Dis. Afr., 5: 431-458.
WHO (1973). Technical report NO. 530.
WHO (1983). RVF, Laboratory diagnostic procedure workshop on RVF held at Aristotation University of Thessalonik, Greece, 6-10 June. Sponsored by WHO, UNDP.

Table (1): Evaluation of local attenuated RVF vaccine

Purity	Identity using DFA	Safety				Potency
		Hamster	Sheep		Cattle)	SNT titer of inoculated sheep S/C (1 field dose) > 40
		I/P injection (0.5 ml of field dose)	S/C injection (25 field dose)	I/V injection (25 field dose)	S/C injection (10 field dose	
Sterile	RVF virus	Safe	Safe	Safe	Safe	Potent

DFA: Direct Fluorescent Assay.
I/P: intra peritoneal.
I/V: intra venous.
S/C: subcutaneous.
SNT: Serum neutralization Test.

Table (2): Evaluation of local attenuated Smithburn RVF vaccine in cattle sera as measured by SNT

Number of animals	SNT titer in cattle post vaccination inoculated with $2X10^{4.5}$ $TCID_{50}$/ml								
	0 days post vaccination	2 Weeks post vaccination	Months post vaccination						
			1	2	3	4	5	6	7
1	2	8	32	64	64	32	32	16	8
2	2	4	16	32	32	16	16	16	4
3	2	8	16	64	64	16	16	16	8
4	2	4	16	32	32	16	16	8	4
5	2	8	32	64	64	32	32	16	8
6*	2	2	2	2	2	2	2	2	2
7*	2	2	2	2	2	2	2	2	2

N.B: * : control animals
Protective titer > 40
SNT: serum neutralization test.

Table (3):Evaluation of local attenuated Smithburn RVF vaccine in cattle sera as measured by ELISA

Number of animal	ELISA titer in cattle sera post vaccination with attenuated RVF vaccine								
	0 day post vaccination	2 weeks post vaccination	Months post vaccination						
			1	2	3	4	5	6	7
1	136	420	910	2221	2200	890	880	620	410
2	132	218	620	890	870	620	615	610	210
3	131	410	620	2220	2210	840	620	610	400
4	120	210	615	910	910	620	620	400	210
5	130	410	920	2210	2200	910	910	610	400
6*	128	130	120	128	130	128	128	130	120
7*	120	132	130	130	130	132	130	130	132

N.B :* :control animals

الملخص العربي

دراسات مناعيه بعد التحصين بلقاح حمي الرفت فالي الحي في العجول

نرمين جوده شفيق و أروي حسن النجارو محمد سعيد واصل

*المعمل المركزي للرقابه علي المستحضرات الحيويه البيطريه- العباسيه- القاهرة.

تـم حقـن لقـاح الرفـت فالي الحي المستضعف عترة سميث بيرن في الأغنام قبل تجربـته فـي العجـول و قـد أثبتـت التجارب أن اللقاح صالح من حيث السلامة و الأمان في الأغـنام و العجـول والهامسـتر الذهبـي أما بالنسبة لاختبار فاعلية اللقاح وجد أن اللقاح بالرغم مـن ثـبوت فاعليته في الأغنام عند استخدامه في تحصين العجول أن المستوى المناعي باستخدام اختـبار الـتعادل فـي السـيرم وصـل أكثر من 40لمدة3 شهور و انخفض المستوى المناعي لمسـتوى أقـل مـن 40 مـن الشهر الرابع إلي السابع وكانت نتائج اختبار الاليزا مطابقة لنتائج اختبار التعادل في السيرم

****