Comparative Evaluation of the Inactivation Efficacy of Different Chemical Inactivating Agents on the Immunogenicity of Rift Valley Fever Viral Vaccine

A thesis

Submitted for the degree of M.Sc in Science

(Microbiology)

By

Noha Emad El-Din Abd El-Razek

B.Sc. Microbiology - 2005

Supervisors

Dr. Sahar Ahmed Shoman

Assistant Professor of Virology, Faculty of Science, Ain Shams University

Dr. Aly Fahmy Mohamed

G.M of Applied Research Sector VACSERA - EgyVac

2011

تقييم مقارن لقدرة المثبطات الكيميائية المختلفة على كفاءة القدرة التمنيعية للقاح حمى الوادي المتصدع

رسالة

مقدمة للحصول علي درجة الماجستير في العلوم

في الميكروبيولوجي

من

نهى عماد الدين عبد الرازق

بكالوريوس علوم جامعة عين شمس- ٢٠٠٥

تحت إشراف

د/ سحر أحمد حافظ شومان

أستاذ مساعد قسم الميكروبيولوجي

كلية العلوم جامعة عين شمس

د/ علي فهمي محمد السيد

مدير عام قطاع البحوث التطبيقية

(فاكسيرا)

2011

Table of Contents

Abstract

This work has been carried out to investigate the effect of Ascorbic acid (AA) and Beta-propiolactone (BPL) as inactivants compared to formalin as a traditional inactivant. Inactivation efficacy of BPL was 4 time faster than formalin to induce complete inactivation of the RVFV, while AA showed the least inactivation efficacy. In the mean timed the ED_{50} values of inactivated vaccines were 0.011 ml, 0.006 ml and 0.0024ml for formalin, BPL and AA respectively. Antibody level was time dependent. CAP-F-RVFV, CAP-BPL-RVFV and CAP-AA-RVFV vaccinated groups showed the highest Ab level compared to that elicited post immunization with F-RVFV, Alum-F-RVFV, BPL-RVFV, Alum-BPL-RVFV, A.A-RVFV and Alum-A.A-RVFV adsorbed vaccines. While, IFN-γ level detected post CAP-AA-RVFV was higher than that induced post Alum-BPL-RVFV and CAP-F-RVFV when evaluated using ELISA. Subcutaneous route of administration was appropriate delivery system for adsorbed vaccine models where the effects of calcium phosphate and aluminium phosphate adjuvants were examined in mice for liver, kidney and spleen toxicity. Results recorded that detected tissue irritation due to calcium phosphate and Aluminium phosphate was ended on the 4^{th} and 8^{th} week post vaccination respectively.

I- Introduction

Rift Valley Fever Virus is an arbovirus responsible for recurrent disease epidemics and epizootics in Africa **(Meegan and Bailey, 1989; Peters and Linthicum, 1994).** Natural vertebrate hosts of RVFV include: Cattle, Sheep, Goats, Camels and Humans. Mosquito species of the *Aedes* and *Culex* genera were reported to be the main vectors in Kenya and Egypt, respectively **(Gonzalez-Scarans and Nathanson, 1996; Lubroth *et al.,* 2007).**

RVFV is a member of *Bunyaviridae* family, *Phlebovirus* genus **(Jansen Van Vuren *et al.,* 2007; Murphy *et al.,* 1995)**, the family member are spherical enveloped viruses, its genome consists in three negative single stranded RNA segments referred as L, M and S respectively for Large, Medium and Small. The L-segment codes for the L-protein which is the viral polymerase. The M-segment codes for glycoproteins G1 and G2 and two others proteins of 78 and 14 K. the S-segment codes for the nucleoprotein N and the non-structural NSs protein using an ambisense strategy **(Bouloy, 1991; Elliott et *al.,* 1991; Giorgi, 1996; Schmaljohn, 1996).**

RVFV was first isolated in 1930 near lake Naivasha in Kenya by **Daubney *et al*. (1931)**. Since then, the virus has been shown to be widespread in Sub-Saharian Africa and in Egypt **(Meegan and Bailey, 1989)**. Major epidemic / epizootics occured in Egypt in 1977 (200,000 humans infections and 600 deaths) and in 1993, Mauritania in 1987 (200 humans deaths), Madagascar in 1991 and in Eastern Africa (89,000 infections and more than 500 deaths reported so far) with the last recent outbreak in 1997- 1998 in Kenya, Tanzania, Somalia **(Thonnon *et al.,* 1999)**. In September 2000, a Rift Valley Fever outbreak was reported in Saudi Arabia and Yemen. This was the first cases identified outside Africa **(USACHPPM, 2005)**.

Epizootics of Rift Valley Fever in Africa occurred often when unusually heavy rainfall was observed. Virus circulates among infected arthropod vectors and mammalian hosts particularly cattle and sheep, which represents the most significant livestock amplifiers of RVFV. The virus appears to survive the dried eggs of *Aedes* mosquitoes **(Linthicum *et al.,* 1985)** and can persist in mosquitoes eggs until the next period of heavy rainfall when they hatch and yield RVFV infected mosquitoes **(CDC, 2004; OIE, 2004).**

RVFV infections in livestock are characterized by an acute hepatitis, abortion and high mortality rates, particularly in young animals. While humans infected with RVFV typically develop a mild self limited febrile illness, with retinal degeneration, severe encephalitis, fatal hepatitis and hemorrhagic fever may also occur **(Swanepoel and Coetzer, 2004).**

Vaccines have been the principle means used to control RVFV. Two types of vaccines have been described:

Formalin inactivated RVF vaccines which have been used to immunize animals, laboratory workers, veterinarians and other people at high risk of exposure to RVFV. The cost of the vaccine, the requirement for multiple inoculations and the time interval required to mount a protective immune response, all limit its use for veterinary purposes.

Two live attenuated vaccines, the Smithburn vaccine, also referred as Smithburn neurotropic strain or SNS **(Smithburn, 1949)**, and MP12 **(Caplen *et al.,* 1985)** have been developed. The SNS is the only widely available veterinary vaccine but has serious limitations in practical use, because it has been proven to be teratogenic, cause abortions and encephalitis in young lambs **(Lubroth *et al.,* 2007).**

Accordingly, due to that, safe and efficient vaccines for both human and veterinary use are urgently needed. Cheaper chemicals need to be evaluated for their inactivating properties **(Mohamed *et al.,* 1997).** Ascorbic acid which is not inexpensive but also easily available, is found to be suitable for trigging this idea.

Vaccination to protect animal against infectious diseases may be enhanced by using adjuvants that can selectively stimulate immunoregulatory responses. One of these adjuvants is calcium phosphate which is a novel adjuvant elicited little or no inflammation at the site of inoculation and induced high titres of antibody **(Ellian *et al.,* 2006 and Emerich and Thanos, 2007).** Alum compounds are the most extensively used adjuvants in licensed vaccines. Although they effectively enhance immune response, they cause severe inflammatory reaction at the injection site and the duration of this inflammation is somewhat long as cited by **Goto *et al.* (1997)** who reported that the local tissue reactions caused by injection of calcium phosphate gel completely ceased by the 4^{th} week, while irritation caused by aluminium hydroxide gel persisted for 8 weeks. Furthermore, the CAP gel is economical, simple to manufacture and is a natural constituent of the animal body **(Ellian *et al.,* 2006 and Zhang *et al.,* 2008).**

The present study aim to evaluate the effect of some inactivantes on the immunogenicity of RVFV. This work was designed to include the following items:

1- Evaluation of the efficacy of different chemical inactivantes and relative inactivation kinetics of RVFV and capabilities post viral treatment.

2- Evaluation of the vaccine efficacy post adjuvation with both Alum and calcium phosphate nano-particles and role of particle size as a vaccine vehicles and relative immune response post subcutaneous administration of vaccines.

3- Finally examination of the histopathological changes post immunization with both Calcium phosphate and Alum as adjuvant.

II- Review of literature

The last 30 years of the 20th century have witnessed a dramatic resurgence or emergence of epidemic arboviral diseases affecting both humans and domestic animals. These epidemics have been caused primarily by viruses thought to be under control such as Dengue, Yellow Fever, or viruses that have expanded their geographic distribution such as West Nile and Rift Valley Fever **(Murphy and Nathanson, 1994 and Lederberg, 1996).**

The word arbovirus is an ecological term used to describe viruses that require a blood-sucking arthropod to complete their life cycle **(WHO, 1985)**. By definition, arboviruses require a minimum of two hosts, a vertebrate and an arthropod. Generally, the virus must produce a level of viremia in the vertebrate host for the arthropod to become infected while taking a blood meal. Arboviruses are taxonomically diverse, belonging to 8 viral families and 14 genera. Most arboviruses of public health importance belong to three families: *Flaviviridae*; *Togaviridae* and *Bunyaviridae* **(Gubler and Roehrig, 1998 and Valerie *et al.,* 2010)**.

The arboviruses are, with few exception, Zoonoses that depend on animal species other than humans for maintenance in nature. The most important reservoir hosts for arboviruses of public health importance are birds or rodents, and the most important arthropod vectors are mosquitoes and ticks **(Gubler, 1997)**.

Arboviruses as a group have a global distribution, but the majority are found in tropical areas where climate conditions permit year-round transmission by cold-blooded arthropods. The geographic distribution of some mosquito vectors and some viruses has expanded globally, accompanied by more frequent and larger epidemics. In other cases, the viruses have been introduced into new geographic regions and have taken advantage of susceptible vertebrate and arthropod hosts to become established and cause major epidemics / epizootics **(Gubler, 2002)**.

1. History of Rift Valley Fever :

A zoonosis may be defined as a disease that is naturally transmitted between humans and animals. The most serious zoonosis are viral, and Rift Valley Fever (RVF) falls into this category **(Peters and Linthicum, 1994)**. Domestic livestock serve as amplifying hosts for the virus, which serve in turn to

infect more mosquitoes. The natural vertebrate reservoir host is not known **(Meegan and Bailey, 1988 and Peters, 1997)**.

The natural history of RVF virus is not fully understood. It is not known how the virus was introduced into Egypt or the Middle East, but the ability of RVF virus to be vectored by many different mosquito species, as well as the high viremias which developed in infected animals, results in a pathogen with high potential for geographic spread and maintenance in nature. Until recently, outbreaks of RVF had been confirmed only in sub-Saharan Africa; however, epizootics in Egypt in 1977 and 1993 **(Arthur *et al.*, 1993; CDC, 1994 and Meegan, 1979)** and another in Saudi Arabia and Yemen in 2000 **(CDC, 2000)** have clearly demonstrated the capacity of this pathogen to spread into new regions.

2. Etiology and classification:

The *Bunyaviridae* is a large family of viruses and contains five genera, four of which infect vertebrates, while the remaining genus, *Tospovirus*, contains a group of plant viruses. Three of the vertebrate-infecting genera, *Bunyavirus, Phlebovirus* and *Nairovirus* are associated with arthropods, while the last genus, *Hantavirus*, has no known invertebrate association **(Gerdes, 2004; Tourre *et al.*, 2008).**