Experimental Studies To Evaluate The Administered Dose Of Attenuated Rvf Vaccine In Buffaloe Calves In Egypt

Arwa, H. Elnagar ;* Wassel, M.S.* and Khairat, A. Elian**

*Central Laboratory for Evaluation of Veterinary Biologics, Abbassia, Cairo, Egypt

**Veterinary Serum and Vaccine Research Institute, P.O.B.:131 Abbassia, Cairo, Egypt

ABSTRACT

The present work was designed to detect the most suitable dose of the live attenuated Smithburn Rift Valley Fever (RVF) vaccine in buffaloes. Each of three different doses of such vaccine ($1\times10^{4.5}$; $10\times10^{4.5}$ and $100\times10^{4.5}$ $TCID_{50}$/dose) was inoculated S/C in a group of 2 buffalo calves. Unvaccinated buffalo calve groups was kept control without vaccination. All vaccinated animals did not show any abnormal clinical signs allover the experimental period (6 months). Buffalo calves receiving doses of $10^{4.5}$, $10^{5.5}$ and $10^{6.5}$ $TCID_{50}$/dose exhibited similar titers of serum neutralizing antibody and ELISA titers. Monitoring the antibody titers, we could say that using attenuated Smithburn RVF vaccine with a dose of $10^{4.5}$ to vaccinate buffalo calves give short duration of protection (4months).

INTRODUCTION

Rift Valley Fever (RVF) an acute infectious disease causing mortality in farm animals and humans and recorded for the first time in Egypt during 1978 as it caused high economic losses in young calves, lambs and death in humans *(1)*. The seroepidemiological studies revealed that abortions in buffalo were seen concurrently and antibodies to RVFV were present in 39% of domestic livestock, presumably unvaccinated. RVFV was isolated from an aborted water buffalo fetus *(2)*.

Some reports revealed that Rift Valley fever virus is an arthropod-borne Phlebovirus endemic in sub-Saharan Africa. Large epizootics occur at irregular intervals in seasons of above-average rainfall with persistent flooding and the appearance of large numbers of floodwater-breeding Aedine mosquitoes. The virus is transmitted transovarially and can remain dormant in mosquito eggs during dry interepizootic periods. The disease is characterized by abortion in pregnant animals and a high mortality in newborn lambs, kids, and calves. Susceptibility to disease is related to age and breed, with severe disease occurring in the young of exotic sheep and cattle breeds *(3)*.

Antigenicity of the neurotropic strains of RVF virus was much lower when compared with the pantropic strains. However they remained able to protect sheep against challenge with the pantropic virus for a period of 5 months, following the passive transfer of specific antibody *(4)*. This important observation demonstrated the value of vaccination strategic of non pregnant animals.

The antibody response of cattle to a primary injection of either live attenuated or inactivated RVF vaccines was poor when measured by the serum virus neutralization test (SNT) and the haemagglutination-inhibition test (HI) but a booster dose of inactivated vaccine evoked a good anamnestic response in cattle previously injected with either of these vaccines *(5)*.

The live attenuated vaccine of RVF MP-12 may be safe and efficacious for pregnant or lactating bovids, and a minimal dose of vaccine may provide suitable protection against viremia. RVF MP-12 was safe, immunogenic, and protective in young calves as 2 days of age *(6)*.

Different Egyptian fat-tail sheep were vaccinated with live attenuated and inactivated Smithburn RVF vaccines and it was found that the live vaccine can be safely used to immunize Egyptian fat-tail sheep. The immunogenicity, safety and requirement for only a single inoculation suggested the attenuated Smithburn vaccine may be an effective and practical alternative to the killed

vaccine for use in non pregnant animal vaccination programs *(7).*

The antibody response to vaccination with the live attenuated local Smithburn strain of RVF virus had occurred in some, but not all the cows and buffaloes. Virus isolation from the fetus suggests in utero transmission of used vaccine virus, which resulted in high abortions in European cows *(7, 8).*

The present work was designed to determine the most suitable dose of the live attenuated RVF vaccine that induces the highest antibody level in buffalo calves.

MATERIAL AND METHODS

RVF live attenuated vaccine

A valid locally produced live attenuated Smithburn Rift Valley Fever vaccine was used for both vaccination and serological tests. It was supplied by Rift Valley Fever Research Department: Veterinary serum and Vaccine Research Institute (VSVRI). Abbassia, Cairo, Egypt.

RVF antigen and antiserum

Purified reference RVF antigen and antiserum were kindly supplied by VSVRI.

Horse radish peroxidase conjugate

Rabbit anti-bovine IgG conjugated with horse radish peroxidase No.F7414 was supplied by Sigma Immunochemical Company and used in ELISA.

Experimental design

Seven apparently health susceptible buffalo calves of about 1 year old were kept under observation for a week before vaccination. General clinical examination was carried out and serum samples were collected for detection of RVF antibodies using serum neutralization test. These animals were found susceptible and free from RVF antibodies and divided into 4 groups, (two) animals for each of the first 3 groups and one animal for the last group. Each buffalo; in the first three groups was injected with 1x (contain 10^4-$10^{4.5}$ log10 TCID50) which is the recommended dose for vaccination or 10 xs or 100xs *(9)* "$10^{4.5}$ $TCID_{50}$" S/C while the 7th animal was kept without vaccination as control. All animals were kept under hygienic conditions receiving balanced diet and adequate water. Serum samples were collected weekly for 3 successive weeks and then monthly up to 6 months post vaccination to follow up the induced RVF antibodies using SNT and ELISA.

Virus titration

Titration of RVF virus was carried out according to Walker *(10).*

Serum neutralization test (SNT)

SNT was carried out following the method described by WHO *(11).*

Enzyme immune sorbent assay (ELISA)

It was carried out as previously described *(12).*

RESULTS AND DISCUSSION

Rift Valley Fever (RVF) is one of the most important viral disease representing a great hazard facing both animal and human being populations. Safe and potent vaccines are needed to overcome and eradicate such disease. Many generations of live and inactivated vaccines were developed and many works were carried out in trials to evaluate them to choose the best, but some findings came to recommend the use of live vaccines while others support the use of inactivated vaccines and refuse the live one. So the present study was a trial to investigate the most suitable dose of the live Smithburn vaccine that could be used safely to immunized buffalo calves.

The obtained results of SNT and ELISA (Tables 1&2) showed that all buffalo calves vaccinated with different doses exhibited detectable specific antibodies by the 1st week post vaccination recording their protective titers by the 4th week and still protective up to 4 months then began to decline by the 5th month post vaccination. However, the doses of $1\times10^{4.5}$; $TCID_{50}$/ $10\times10^{4.5}$ and $100\times10^{4.5}$ $TCID_{50}$/dose induced

similar antibody response (64 for SNT and 2120 for ELISA). The obtained results showed that all the used doses were safe and potent where the vaccinated animals did not show any signs of illness and the titers of induced antibodies were found to be protective where it was detected to be >40 coming in agreement with WHO ***(10)*** **and OIE** ***(9)***. Also the present findings appear to be supported by previous several investigations ***(5, 6, 8, 13)*** which stated that the live RVF vaccine was safe and immunogenicly poor for buffalo calves. We did not apply challenge to avoid spread of virus infection as recommended by general organization of veterinary service rules.

So, we could say that using attenuated Smithburn RVF vaccine to vaccinate buffaloes give short duration of protection (4months) and no difference between using normal dose ($1\times10^{4.5}$ $TCID_{50}$/buffaloe) and higher dose ($10\times10^{4.5}$ $TCID_{50}$ or $100\times10^{4.5}$ $TCID_{50}$/buffaloe) in vaccination. Also it is important to revaccinate buffaloes every 4 months.

Table 1. Mean RVF neutralizing antibody titers in buffalo calves vaccinated with live attenuated Smithburn vaccine

The used dose	Mean titer of RVF neutralizing antibodies/ periods post vaccination								
	1WPV*	2WPV	3WPV	4WPV	2MPO**	3MPV	4MPV	5MPV	6MPV
$1\times10^{4.5}$ $TCID_{50}$	8	16	32	64	64	64	64	32	16
$10\times10^{4.5}$ $TCID_{50}$	8	16	32	64	64	64	64	32	16
$100\times10^{4.5}$ $TCID_{50}$	16	16	32	64	64	64	64	32	16
Control	0	0	0	0	0	0	0	0	0

*WPV= Week post vaccination **MPV= Month post vaccination
N.B.: The protective level of RVF neutralizing antibody titer is >40.

Table 2. Mean RVF ELISA antibody titers in buffalo calves vaccinated with live attenuated Smithburn vaccine

The used dose	Mean titer of RVF neutralizing antibodies/ periods post vaccination								
	1WPV*	2WPV	3WPV	4WPV	2MPO**	3MPV	4MPV	MPV	6MPV
$1\times10^{4.5}$ $TCID_{50}$	410	840	1600	2100	2190	2200	2210	400	200
$10\times10^{4.5}$ $TCID_{50}$	480	840	1620	2120	2220	2240	2220	400	220
$100\times10^{4.5}$ $TCID_{50}$	460	840	1620	2120	2220	2210	2210	410	210
Control	120	130	132	120	131	131	130	132	128

*WPV= Week post vaccination **MPV= Month post vaccination

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الملخص العربى

دراسة معملية لتقييم جرعة لقاح الرفت فالى الحى المستضعف المحقونة فى العجول الجاموسى فى مصر

أروى حسن النجار*- محمد سعيد واصل*- خيرات عبد المجيد عليان**

*المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية
**معهد بحوث الأمصال واللقاحات البيطرية
العباسية- القاهرة-ص. ب: ١٣١

أجريت هذه الدراسة لتحديد أنسب جرعة من لقاح حمى الوادى المتصدع عترة سميث بيرن الحى المستضعف لتحصين العجول الجاموسى فى مصر حيث تم تحصين ثلاث مجموعات من الجاموس بثلاث جرعات مختلفة من اللقاح $1\times10^{4.5};10\times10^{4.5}$and $100\times10^{4.5}TCID_{50}$ مرة واحدة تحت الجلد. هذا وقد وجد أن اللقاح المستخدم آمن حيث لم تظهر أية أعراض مرضية على الحيوانات المحصنة. وقد وجد أن الجرعات $10\times10^{4.5}$; $100\times10^{4.5}TCID_{50}$ تعطى قيما متماثلة لأختبار المصل المتعادل والأنزيم المرتبط المناعى من الأسبوع الرابع حتي الشهر الرابع بعد التحصين ثم تبدأ فى النقصان تدريجيا من الشهر الخامس بعد ذلك. وقد لوحظ أن هذه المعايير ذات قيمة وقائية تكفى لحماية الجاموس ضد المرض لفترة قصيرة وينصح بإعادة التحصين بعد أربعة أشهر.