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Trials for Local Production of Rift Valley Fever Vaccine From Inactivated Smith Burn Strain

Thesis presented by

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(Infectious diseases)

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ABSTRACT

The vaccination campaign (using live Smith burn virus vaccine) performed in Egypt during the outbreaks of 1996,1997 and 2003 did not stop the disease but it causes viremia in vaccinated animals which explain the fear of spread of the virus via mosquito's vectors and the concern possible reassortment event with wild type viruses. The Egyptian GOVS in the 2008 demonstrated that (Rift Valley Fever) live vaccine (Smith burn strain) isn't used in Arab Republic of Egypt in the present time. Consequently, the RVF vaccination programs in Egypt are performed by inactivated (ZH-501) RVF vaccine. In this study Inactivated smith burn RVF vaccine was prepared and evaluated by serological tests (SNT and ELISA), compared with local vaccine. Results indicated that inactivated Smith burn RVF vaccine was more protective, induced high titer of antibodies. Furthermore, time of protection is longer than the local vaccine.

Keywords:

RVF, Vaccine, Evaluation, Smith burn, ZH-501.

A-Enzootic cycle of RVF virus:

Rift Valley fever virus survives in enzootic regions of Africa in a silent infection cycle and emerges after periods of rainfall, to initiate disease epizootics ***Figure (2).*** It is local transmission cycle between mosquitoes and animal host.

B- Epizootic -Epidemic cycle of RVF virus:

It was discovered in 1980 that the virus is transmitted transovarially among floodwater Aedes mosquitoes ***(Gerdes, 2002***). The virus survives for very long periods in mosquito eggs laid at the edges of usually dry depressions, called "dambos," which are common throughout grassy plateau regions. When the rains come and the dambos flood, the eggs hatch, and infected mosquitoes emerge and infect wild and domestic animals.

In an epizootic, virus is amplified in wild and domestic animals by many species of Culex and other Aedes mosquitoes ***(James and Edward, 2011).*** These mosquitoes increase in number after heavy rains or when improper irrigation techniques are used; they feed indiscriminately on viremic sheep, cattle and humans .

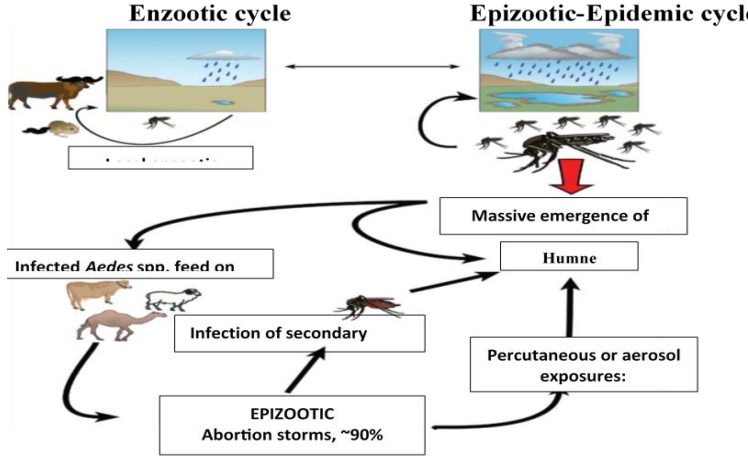


Figure (1): Enzootic and epidemic-epizootic transmission cycles of Rift Valley Fever (RVF) virus. In the enzootic transmission cycle (top left panel), wildlife (e.g., African buffalo species) are potential maintenance hosts. In the epidemic-epizootic transmission cycle (remainder of figure), livestock amplification hosts and secondary bridge vectors are involved *(James and Edward, 2011).*

2.2.3. The role of camels in RVFV transmission

Camels are multipurpose domestic animals used for meat, hair and hide production beside transportation Also, camels play certain role in the continuous introduction of RVFV in Egypt. The continuous importation of camels, from the Sudan was the main source of infection in RVF outbreaks in Egypt ***(Abdel Rahim et al., 1997***), However, the importation from Africa consider of high risk due to the presence of trans-boundary animal diseases such as Rift Valley Fever and Foot and Mouth Disease, with the absence of an effective traceability system that acts as proxy for quality assurance ***(Matete et al., 2010***).

Widespread abortion waves in camels were observed during RVF outbreaks in Kenya and Egypt. Camels are playing a major role in the spread of RVF from northern Sudan to southern Egypt in 1977. Additionally RVF virus was previously isolated from blood samples from healthy, naturally infected camels in Egypt and Sudan. Camels probably played a central role in the local amplification of the virus(***Ould El Mamy Ab et al.*** **2011).**

2.3. Host susceptibility:

Rift Valley Fever has wide range of hosts, Domesticated ruminants such as sheep, cattle, and goat are the predominant hosts and it was early established that the newborn lamb was particularly susceptible **(Daubney R *et al* ., 1931).**

Pigs failed to react to experimental infection with a low virus dose but higher doses caused viremia. **(Scott, 1963).**

Although an experimentally infected horse have shown to be resistant , the virus has been isolated from horse, donkey, and camels during an outbreak. **(Imam *et al* ., 1979).**

mortality and abortion rates vary from (5% -100%) in different outbreaks and between different flocks. The mortality rate in cattle is usually less than 10% ***(OIE, 2014).***

Livestock species and age within species shows marked biological heterogeneity in terms of disease outcomes ***(Swanepoel and Coetzer, 2004).*** Thus, young lambs (<1 month old) are highly susceptible to RVFV infection, with case fatality rates (CFR) reaching 90 to 100%. Adult sheep are less susceptible to infection, with CFR of approximately 10 to 30%. However, abortion rates are very high (90 to 100%). Neonatal (<1 month old) calves are less susceptible than neonatal lambs with CFR ranging between 10 and 70%. Adult cattle are more resistant than adult sheep with CFR of approximately 5 to 10%. Although goats are highly susceptible to infection, they appear to be more refractory to severe disease than sheep ***(Bird et al., 2009).***

2.4.2Viraemia development and general immune response:

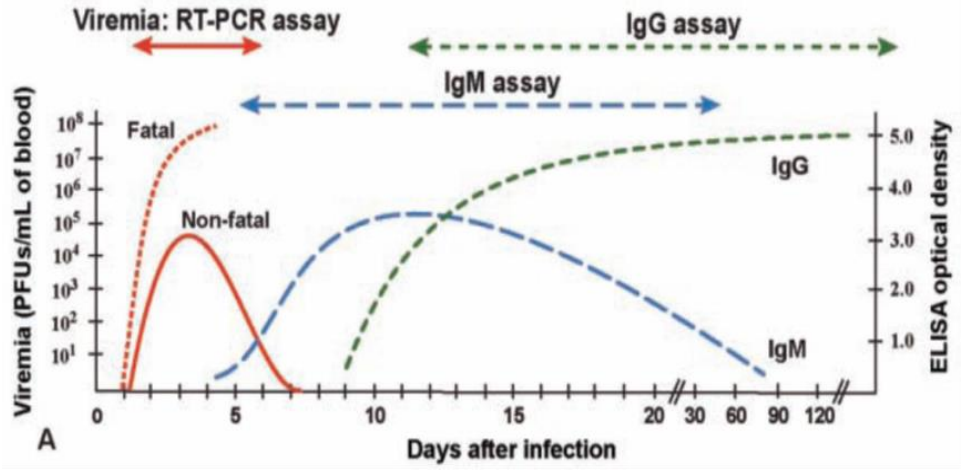
Rift Valley fever virus has a high ability for development of significant viraemia in sheep, goats and cattle (1.0×10^6 to 1.0×10^8 PFUs/mL of blood) ***(Bird et al., 2009).*** The vertebrate hosts are viraemic for only 2–7 days ***(Pepin et al., 2010)*** implying that they are unlikely to serve as long-term reservoir of the virus. Viraemia development and intensity appears to be age and species dependent. For example, viraemia in lambs that are less than one week old is detectable within 16 hours of infection that has been initiated with small doses of the virus, and persists for the duration of infection that mostly ends fatally within (36- 42) hours. In older ruminants, viraemia develops more slowly becoming detectable (1 – 2) days post infection. In adult vertebrate hosts, viraemia is most intense on the second to fifth day ***(EFSA, 2005).***

Following RVFV infection, adaptive immune response is developed, with the production of detectable neutralizing antibodies from the 4th – 8th day after infection ***(Morrill et al., 1987).*** These antibodies are accompanied with

production of Immunoglobulin (Ig) M and IgG antibodies. As in other infections, the detection of RVF IgM antibodies denotes recent RVFV infection. Immunoglobulin M antibodies do not persist beyond the 50th to 90th day in the majority of cases after infection ***(Bird et al., 2009; Pepin et al., 2010).***

Generalized time course of viraemia and antibody response against RVFV in livestock. Note that viraemia levels attained determine the prognosis of a case ***(Bird et al., 2009).***

Figure (2) illustrates a generalized time course of viraemia and antibody response against RVFV in livestock.



2.4.3. Post mortem lesions:

RVF causes petechial haemorrhages in the serous surfaces and yellow necrotic foci in the liver of lambs died from RVF, **(Abou Zaid et al., 1996)**.The most sever lesion occurring in aborted fetuses and newborn lambs is a moderately to greatly enlarged, soft, friable liver with a yellowish brown to

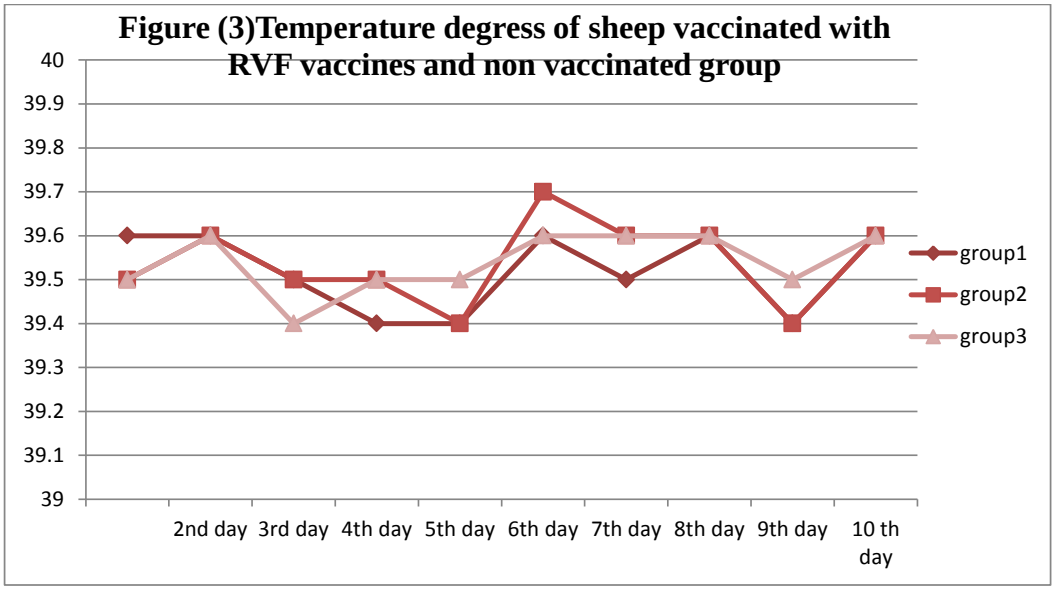


Table (10) Collective results of 10 days monitoring the clinical status of vaccinated sheep with different RVF vaccines :

Clinical status	Inactivated Smith burn RVF vaccine	Inactivated ZH501 Vaccine
Granuloma at site of injection	None	None
Fever	None	None
Mortalities	None	None
Depression and Anorexia	None	None
Diarrhea	None	None
Nasal Discharge	None	None
Respiratory manifestations	None	None

4.6. Evaluation of humoral immune response in vaccinated sheep with different inactivated RVF virus vaccines:

4.6.1. Serum Neutralization test:

The mean neutralizing index in sheep vaccinated with inactivated smith burn RVF vaccine reached above the protective level (1.5) at the 2nd week post vaccination (1.8) and increased gradually till reached the peak (2.4) at 2nd month and continue to 3rd month post vaccination then the level decreased to be (1.5) at the 8th month post vaccination and then decline to a non-protective level .

The mean neutralizing index in sheep vaccinated with inactivated (ZH-501) RVF vaccine reached the protective level (1.5) at the 2nd week post vaccination (1.5) and increased gradually till reached the peak (2.2) at the 3rd month post vaccination then the level decreased to be (1.5) at the 7th month post vaccination and then decline to a non-protective level **.**

Table (11): RVF Neutralizing antibody indices of sheep vaccinated with RVF vaccines and non-vaccinated group

Animal group	No.of animals	Before Vaccine.	Weeks post vaccination				Months post vaccination										
			1st w.	2nd w.	3rd w.	4th w.	2nd m.	3rd m.	4th m.	5th m.	6th m.	7th m.	8th m.	9th m.	10th m.	11th m.	12th m.
Gp. 1 vaccinated with inactivated smith burn RVF vaccine	4	0.4	0.6	1.8	2.1	2.1	2.1	2.1	1.8	2	1.8	1.5	1.5	1.2	0.9	0.9	0.3
		0.3	1.3	1.8	1.8	2.2	2.4	2.4	2	1.8	1.8	1.5	1.2	1.2	1.2	0.9	0.6
		0.5	1.5	1.8	1.8	2	2.7	2.4	2	2	2	2	1.8	1.5	0.9	0.6	0.6
		0.3	0.6	1.8	1.8	2.1	2.4	2.7	2.4	2.1	2.1	2.1	1.5	1.5	1.5	1.2	0.9
	mean	0.4	1	1.8	1.9	2.1	2.4	2.4	2.1	2	1.9	1.7	1.5	1.3	1.1	0.9	0.6
Gp . 2 vaccinated with inactivated RVF vaccine(ZH-501)	4	0.4	0.6	1.8	1.8	1.8	1.8	2.1	2.1	2.1	2	1.8	1.3	1.1	0.9	0.9	0.8
		0.3	0.3	1.2	1.5	1.8	2.1	2.1	2	1.5	1.5	1.3	1	0.7	0.5	0.5	0.4
		0.5	1.5	1.8	1.8	2.1	2.1	2.2	1.8	1.8	1.8	1.5	1.3	0.9	0.6	0.6	0.5
		0.3	0.3	1.2	1.5	1.8	1.8	2.2	2	1.8	1.5	1.5	1.3	1	0.9	0.9	0.8
	mean	0.4	0.6	1.5	1.6	1.9	2	2.2	2	1.8	1.7	1.5	1.2	0.9	0.7	0.7	0.6
GP.3 Non vaccinated group	3	0.2	0.1	0.3	0.4	0.3	0.2	0.3	0.4	0.3	0.3	0.3	0.2	0.2	0.2	0.3	0.3
		0.1	0.2	0.1	0.2	0.1	0.3	0.1	0.2	0.3	0.2	0.4	0.1	0.1	0.3	0.2	0.2
		0.1	0.2	0.3	0.2	0.1	0.3	0.4	0.5	0.2	0.3	0.5	0.1	0.1	0.2	0.1	0.2
	mean	0.1	0.1	0.2	0.3	0.2	0.3	0.3	0.4	0.3	0.3	0.4	0.1	0.1	0.2	0.2	0.2

Protective neutralizing index is 1.5

The mean protective level for the neutralizing antibody indices is (1.8) for inactivated smith burn RVF vaccine and (1.5)for (ZH-501)RVF vaccine according to *Randall, et al 1964*)

Figure (4)Neutralizing antibody indices of sheep vaccinated with RVF vaccines before vaccination till one month post vaccination

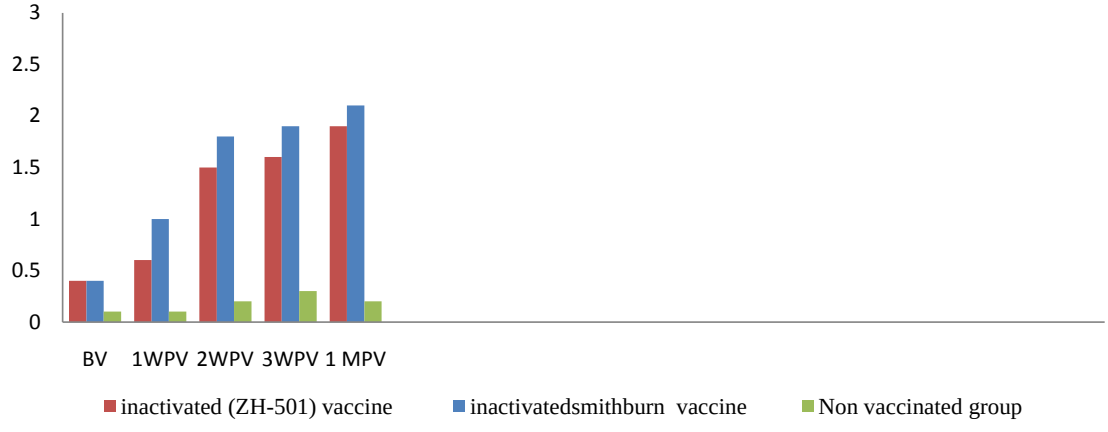
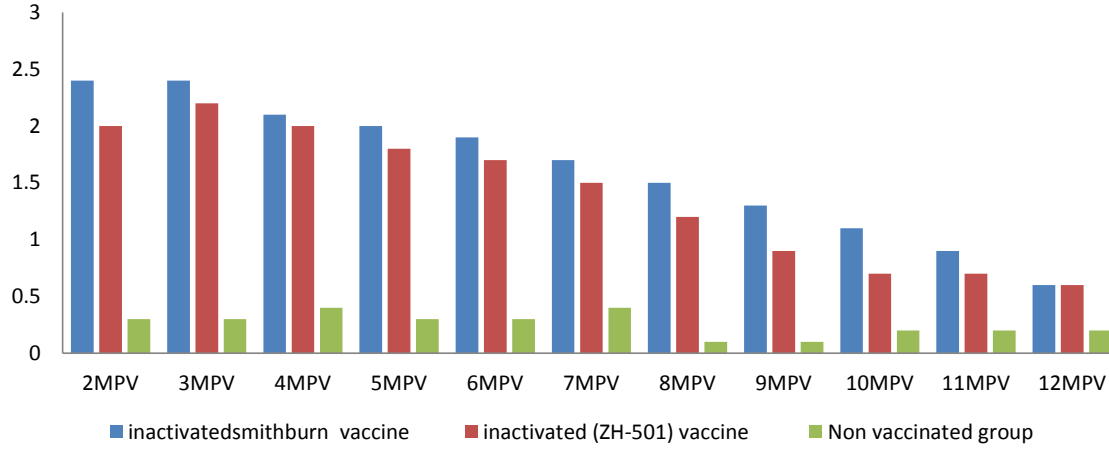


Figure (5) Neutralizing antibody indices of sheep vaccinated with RVF vaccines from 2nd month till 12 month post vaccination



Bv : Before vaccination ***Wpv** :week post vaccination ***Mpv** :month post vaccination .

Figure (6) ELISA Optical Density of sheep vaccinated with RVF vaccines before vaccination till one month post vaccination

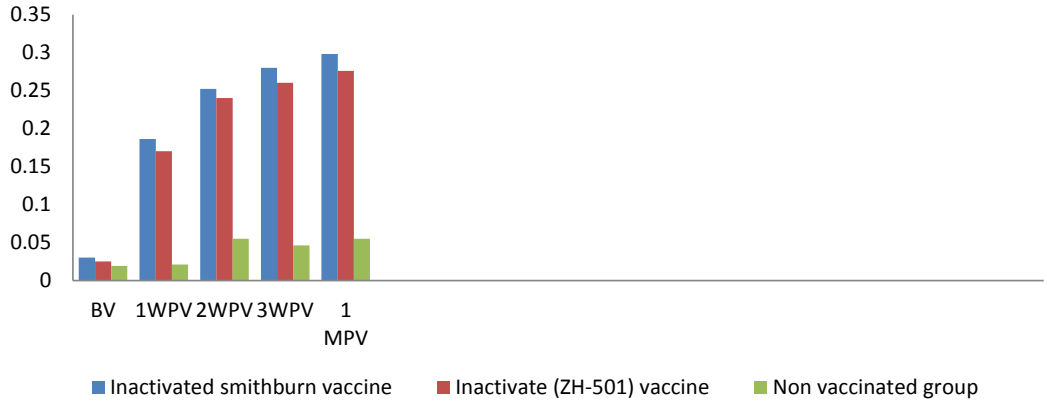
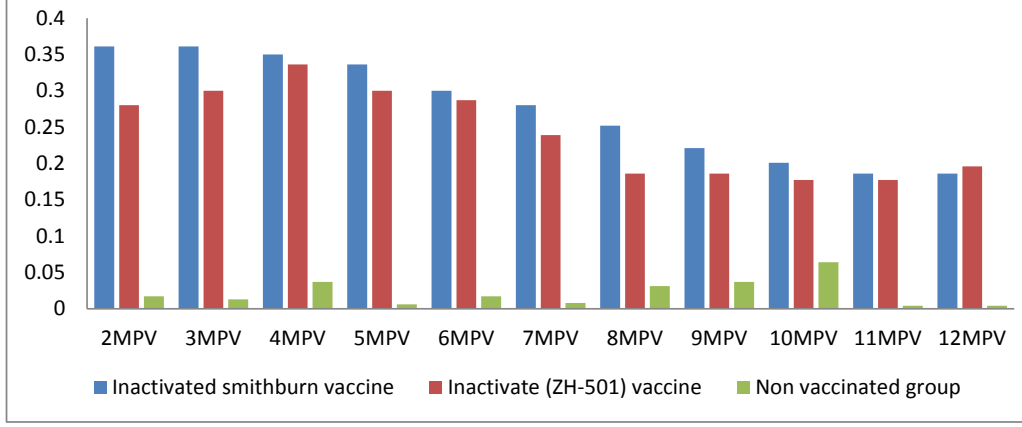


Figure (7) ELISA Optical Density of sheep vaccinated with different RVF vaccines from 2nd month till 12 month post vaccination



Bv: Before vaccination ***Wpv** :week post vaccination ***Mpv** :month post vaccination .