

Seroprevalence of Rift valley fever, in sheep and humans, Seedy Salem (2003)

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Elevated rectal temperature, mucopurulent nasal discharge, mortalities and abortion of pregnant ewes were the main clinical abnormalities recorded in sheep flocks at Seedy Salem Village, Kafr Al-Sheikh Governorate, Egypt following their vaccination by the attenuated Rift Valley vaccine. Meanwhile, 45 of the farmers in the village showed variable findings suggestive for a RVF epidemic and 17 farmers died of haemorrhagic fever as reported by the World Health Organization, WHO (2003). Blood serum samples were collected from 78 humans and 86 sheep and tested for RVF antibodies. We found that, where 11 (12.94%) and 10 (19.30%) of sheep and human sera respectively were positive. The recent history of vaccination of such flocks using the attenuated RVF vaccine lead us to suggest that the used vaccine might be the source of such epidemic.

RVF is an infectious arthropod-borne viral zoonotic disease that seriously affects domestic livestock, particularly sheep and cattle, as well as humans. The disease is classified as a List A disease by the Office International des Epizootics (OIE), Barnett *et al.* (1996) owing to its major socioeconomic and public health importance, rapid spread even across national borders and its great potential for international trade of animals and animal products.

The disease in human is manifested by variable signs including asymptomatic infection, benign febrile illness and severe illness that can include retinitis, encephalitis, and fatal hemorrhagic fever (Laughlin 1979). Infected sheep commonly develop fever, mucopurulent nasal discharge and abortion; the most prominent sign, in pregnant ewes. High mortality, up to 90%, is observed among lambs especially newly born ones those under one week of age, (Buisen and Hyde 1998). RVF is caused by an arthropod-borne member of the genus *Phlebovirus*, family *Bunyaviridae*, (Eddy and Peters, 1980).

During the subsequent decades, Rift Valley fever exclusive epizootics associated with human epidemics were reported in sub-Saharan Africa, (Peters and Linthicum, 1994) including South Africa. (Mundel and Gear 1951), Chad and Cameroon (Maurice 1967), Niger (Mariner *et al.*, 1995) and Madagascar (Morvan *et al.*, 1991).

Recurrent viral activity of RVF that occurs in localized areas in southern and eastern Africa, during most outbreaks might account for the survival of the virus during inter-epidemic periods, FAO (2000). Moreover, the inter-epizootic survival of RVF virus is believed to be due to survival of infected *Aedes* eggs in the dry mud for several seasons until the next heavy rainfall where they hatch infected mosquitoes, (Linthicum *et al.*, 1985).

Outbreaks of RVF were restricted to the sub-saharan Africa until two outbreaks occurred in Sudan 1976, (Eisa *et al.*, 1980) and Egypt 1977, (Meegan *et al.*, 1979), reflecting the ability of RVF virus to transmission irrespective to the national borders, (Barnett *et al.*, 1996). The first report of RVF outside Africa was in Saudi Arabia and Yemen 2000 as confirmed by Centers for Disease Control and prevention, (CDC 2000). It was thought that the virus was introduced through importation of infected livestock from Africa or through wind born infected mosquitoes, (Madani *et al.*, 2003).

The means of RVF introduction to Egypt have been thought to be due to transportation of living viremic animals through Nile or introduction of infected mosquitoes in planes or by wind movement to Aswan high dam area, where agricultural development provided irrigation channels highly suitable for the breeding of *Culex*

pipens; the vector of the disease in Egypt, FAO (2000).

It had been thought that the use of incomplete inactivated vaccine was the source of the virus and the cause of reemergence of RVF in Egypt, (Peters, 1997 and Abd el-Rahim *et al.*, 1997).

Few days after vaccination of sheep flocks using attenuated RVF vaccine in Seedy Salem village, Kafr Al-Sheikh Governorate, Egypt, vaccinated sheep showed elevated rectal temperature, mucopurulent nasal discharge, lamb mortalities and abortion of pregnant ewes. Meanwhile, 45 of the farmers in the village showed variable findings suggestive for RVF. 17 human cases died of hemorrhagic fever as reported by the world health organization (WHO 2003).

The association of recent sheep vaccination by the attenuated RVF vaccine will the suspected of RVF signs suggestive for RVF epidemic in both sheep and humans motivated us to direct the aim of the present work for serodiagnosis of suspected RVF diseased human and sheep as well as to investigate the probable source of infection.

Material and Methods

Animals. A total of 286 sheep at Seedy Salem village, Kafr Al-sheikh comprising 5 (A-E) flocks were employed in this study.

Blood serum samples. Blood was collected from a total of 85 sheep previously vaccinated using attenuated RVF vaccine, (Sera and Vaccines Research Institute, Abbasia, Egypt) under supervision of the veterinary authorities. These animals represented the above-mentioned five flocks. Blood samples were also collected from 78 humans (52 farmers that were in contact with diseased sheep and 26 individuals of other occupations including hospitalized persons). Serum samples were separated, coded and stored at -70°C.

Virus. RVF was kindly supplied by Veterinary Sera and Vaccines Research Institute, Abbassia, Cairo, Egypt.

Clinical examination. Sheep was carried out according to (Radostits *et al.*, 2000).

Virus neutralization test. (VNT) was carried out according to (Walker *et al.*, 1970).

Results and Discussion

Table (1): Results of the clinical examination of sheep flocks

Flock	Number Of Sheep	Diseased sheep				Apparently healthy sheep
		Fever	Abortion	Death	Morbidity	
A	38	30 (78.94%)	8 (21.05%)	6 (15.78%)	30 (78.94%)	8 (21.05%)
B	42	34 (80.95%)	11 (26.19%)	7 (16.66%)	34 (80.95%)	8 (19.04%)
C	57	44 (77.19%)	13 (22.80%)	9 (15.78%)	44 (77.19%)	13 (22.80%)
D	63	44 (69.84%)	14 (22.22%)	11 (17.46%)	44 (69.84%)	19 (30.15%)
E	86	57 (66.27%)	10 (11.62%)	7 (8.13%)	57 (66.27%)	29 (33.72%)
Total	286	209 (73.07%)	56 (19.58%)	40 (13.98%)	209 (73.07%)	77 (26.92%)

Table (2): VNT of human sera.

Occupation	Number of individuals	SNT positive
Farmers	52	10 (19.30%)
Other occupations	26	-
Total	78	10 (12.82%)

Table (3): VNT titers of positive human sera.

SNT titer	Number of samples
1/4	3/10 (30%)
1/2	4/10 (40%)
Less than 1/2	3/10 (30%)

Table (4): VNT of sheep sera.

Sign	Number of sheep	SNT positive
Abortion	36	9 (25%)
Non pregnant ewes (fever)	22	1 (4.54%)
Rams (fever)	15	1 (6.66%)
Apparently healthy	12	-
Total	85	11 (12.94%)

Table (5): VNT titers of positive sheep sera.

SNT titer	Number of samples
1/8	2/11 (18.18%)
1/4	2 (18.18%)
1/2	3/11 (27.27%)
Less than 1/2	4/11 (36.36%)

The antibody titers were calculated according to (Singh *et al.*, 1967).

Clinical examination of 286 RVF recently vaccinated sheep in five sheep flocks (Table 1), revealed fever associated with inconsistent mucopurulent nasal discharge and/or diarrhea in 209 (73.07%), abortion of pregnant females in 56 (19.58%) and death of 40 (13.98%).

Meanwhile, 45 of the farmers in the village showed variable degrees of illness ranging from fever to severe rhinitis and headache and 17 died of hemorrhagic fever.

Such epidemic form that involved sheep and humans as well as the history of recent vaccination of sheep using the attenuated RVF vaccine was suggestive for RVF epidemic. The high morbidity rates among sheep flocks that ranged from 66.27% to 80.95%. (Table 1) disagreed with the expected morbidity for a mosquito-borne disease as RVF that seldom exceeds 30% (Barnett *et al.*, 1996) in adult sheep that almost constituted the 5 flocks under investigation. However, these results increased the suspicion of the role of the attenuated vaccine as a source of infection.

It is worth mentioned that, this outbreak occurred in August whereas mosquito population usually increases in summer, (Abdel-Rahim *et al.*, 1997). Yet, the disease was restricted to Seedy Salem district and no cases were reported even in the nearby villages either in human or animals indicating the absence or minimal role of mosquitoes in this localized outbreak.

Mortality and abortion among sheep, (Table 1) were (8.13%-17.46%) and (11.62%-26.19%) respectively, percentage were not high due to the expected low number of lambs and pregnant ewes in this period where it was not a lambing season where mortalities among newly born lambs might be above 90%, (Buisch and Hyde 1998).

Concerning sheep sera, RVF antibodies could be detected in 11(12.94%) of the collected samples where nine (25%), one (4.54%) and one (6.66%) of serum samples of aborted ewes, non pregnant females and rams respectively were positive, (Table 4). although, RVF antibodies could not be detected in the majority of diseased sheep, this does not exclude the infection rather than being a failure of antibodies to develop to a detectable level that usually occurs four to six days postinfection (Paweska *et al.*, 2003).

Using VNT, (Table, 2), 10 (19.30%) out of 52 of farmers' blood sera were positive for RVF antibodies. This was an evidence of their exposure to infection and accounts for the development of the signs contrary to persons of other occupation where 26 (100%) sera were VNT negative. Such definite findings were parallel to those reported by McIntosh *et al.*, (1980) who stated that even in major outbreaks of RVF; most patients are usually farmers, veterinary surgeons or meat dealers who are highly exposed to the virus that is present in high concentration in the infected tissues at high concentration.

Neutralizing antibody titers of human sera (Table 3), were as low as 1/4-1/2; this might be attributed to early collection of blood samples before the rise of the antibody titer that begins four to six days postinfection, (Paweska *et al.*, 2003). However, the village was kept under quarantine measures and no more samples were available.

The results obtained in this study indicated that the use of attenuated RVF vaccine in Egypt may be risky not only as a cause of abortion, but also as initiator of the epidemic cycle due to the possible insufficient attenuation, (Vialat *et al.*, 2003) or due to accidental vaccination of pregnant ewes. Therefore, properly inactivated RVF vaccine together with continuous surveillance and prevention of importation of animals or meat from enzootic countries should be applied to the control and the monitor of the disease.

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