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# Recent Trends for Diagnosis of Rift Valley Fever in Animals and Mosquitoes in Egypt with Special Reference to the Carrier

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**Abstract:** The ability of the Polymerase Chain Reaction (PCR) to diagnose Rift Valley Fever virus (RVFv) infection in an arthropod vector was used. RFVv RNA was detected in RNA extracts from infected mosquitoes tissue samples by reverse transcription and enzymatic amplification of the resulting cDNA using Taq DNA polymerase, followed by characterization of the amplified product by agarose electrophoresis. Mosquitoes (*Culex sp.*) samples were collected along one year from February 2004 to January 2005 from Kafer El- Sheikh and El-Beheira governorates. Viral RNA in mosquitoes from all months except (Decmber 2004 to January 2005) in Kafer El- Sheikh was detected while in El- Beheira it was detected only in three months period (January, August and September 2004). This indicates that the probability of the transmission of the RVF disease by mosquitoes is higher in Kafer El-Sheikh than in El-Beheira governorate. Enzyme Linked Immuno-Sorbent Assay (ELISA) was used to detect Polycolonal antibodies IgG of RVFv in infected animals. Serum samples from 151 susceptible animals (cattle), 125 contact animals (donkeys) and 97 contact domestic birds, from two governorates; Kafer EL-Sheikh and El- Beheira were examined by using ELISA test. The prevalence rate in Kafer El- Sheikh for those animals were 20.37, 9.54 and 0.85%, respectively, while in El- Beheira governorate, the prevalence rates were 13.15, 5.36 and 0.49%, respectively. This indicated that the prevalence rate of RVF in Kafer El-sheikh was higher than El-Beheira.

**Key words:** RVF virus · PCR · ELISA · Mosquitoes · Animals

## INTRODUCTION

Rift valley fever is acute infectious arthropods borne viral disease primary affecting domestic animals with occasional involvement of man. It is responsible for serious economic losses due to abortion and heavy mortality in young animals [1, 2].

The 1977 Egypt outbreak caused by an unexplained spread from Sudan, probably by imported camels or sheep or by mosquitoes, affected 25 to 50 % of all sheep and cattle. Among humans, 18,000 clinical cases, 598 deaths from hemophilic fever were recorded [3]. The RVF outbreaks in Egypt have cost the live stock industry about U.S $ 82 million during 1977 [4]. It is not clear until now, whether the source of reoccurrence of Rift Valley fever into Egypt in 1993 is it due to carrier animals or infected mosquitoes. Susceptible animals are closely related to humans and surveillance of imported livestock is not systematic [5 ,6].

Enzyme-Linked Immuno-Sorbont Assay (ELISA) was used as a sensitive, quick and accurate test for the detection of antibodies of RVF in the serums of infected animals [7].

Molecular techniques as RT. PCR, for detection of viral RNA of RVFv offer more advantages than virus isolation methods. RT. PCR is more sensitive, reproducible and applicable [8]. It provides a promising option for diagnosis and detection of viral RNA of RVFv in mosquitoes [9].

Therefor the aim of the present study is to investigate the following aspects:

- Application of prevalence survey on the sera of cattle in high risk areas (Kafer EL-sheikh and El- Beheira governorates) by using ELISA test.
- Studying the role of contact donkeys and domestic birds as a reservoir for transmission of the disease to susceptible animals by application of sera ELISA test.

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- Detection of RNA in mosquitoes in infected areas by using RT PCR technique to detect the role of mosquitoes in the transmission of RVFv.

## MATERIALS AND METHODS

**Animals:**

**1- Cattle:** A total of 825 at Risk (460 from Kafer El-sheikh and 365 from El Beheira governorates) with samples size of 151 (96 Kafer El-Sheikh and 48 El-Beheira governorates).

**2- Donkeys:** A total 715 at Risk (398 Kafer El-Sheikh and 317 El-Beheira) and sample size of 125 (85 Kafer El-Sheikh and 40 El. Beheira governorates).

**3- Domestic birds:** A total 560 at Risk (355 Kafer El sheik and 205 El Beheira governorates) and sample size of 97 (67 Kafer El sheikh and El Beheira governorates).

Serum samples were collected from those animals along one year from January 2004 to January 2005 and stored at -20°C, till used for studying the prevalence rate of RVF viral antibody by using ELISA test.

**Collection of mosquito samples:** Mosquitoes were collected from area positioned at ground level near (cattle, donkeys and domestic birds) house at different localities and seasons. The collecting started at January 2004 to January 2005) by means of battery powered miniature light trap [10]. Mosquitoes were identified according to [11]. Pools of 50 mosquitoes were prepared for each month for serological diagnosis of RVFv by using RT. PCR.

**Enzyme Linked Immuno-Sorbent Assay (ELISA):** Anti-animal IgG and reagents–Peroxidase conjugated horse were obtained from Sigma, USA. All other chemicals were obtained from Sigma. The RVF antigen was used and prepared according to [12].

**ELISA procedure:** The test was carried out by the method described by [13]. Serum samples were considered positive if it had an optical density (O.D.) equal to or greater than the cut off point which was calculated as the mean absorbance negative control samples plus 3.29 times of standard deviation [14].

**Extraction of RNA from mosquitoes and inactivated RVF vaccine:** Mosquito's pools (50 mosquitoes) were prepared from each month and then homogenized in mortar by using liquid nitrogen then the Homogenized mosquito pools and inactivated RVF vaccine were taken and the same RNA extraction protocol was applied. The extraction was done using commercial kit (Qiagen, viral RNA Mini kit, cat No. 32904).

**Determination of RNA concentration in samples:** Determination of RNA concentration was carried out by using spectrophotometer, the concentration was calculated as follows:

RNA conc. (ng/ul ) =Optical density at D260nm X (40) dilution factor X (50)Dilution factor = 50.

**Application of RT PCR on RNA samples:** RT –PCR was implemented using RT –PCR kit (Qiagen ) and the following primers sequence

(A) RVF3:5- VAG AIG ACA GGT GCT AGC-3.

(B) RVFv4:5-CT ACC ATC TCC AAT CTT GG-3

according to [9].

Inactivated vaccine of the RVF was used as a control positive, commercially supplied by VACSERA (Holding company for Biological Products and Vaccines– Agouza). Reg. No:16/99. Double distilled water was used as control negative.

RT- PCR products were analyzed using 2.5% agarose gel electrophoresis.

## RESULTS

**Enzyme Linked Immuno Sorbent Assay (ELISA):**

**Cattle:** Prevalence survey on the sera of susceptible animals (Cattle) in infected areas (Kafer–EL-Sheikh and EL–Beheira governorates) was done. One hundred and fifty Serum samples were tested against RVFv from a total of 825 Cattle at risk areas by using ELISA. Results are illustrated in Table 1. The prevalence rate was 20.87 and 13.15% in Kafer EL-Sheikh and EL-Beheira governorates, respectively.

**Contact animals:**

**Donkeys:** Prevalence of RVF viral antibodies in sera of Contact animals (donkey) at (Kafer El-Sheikh and

Table 1: Prevalence of RVF viral antibodies in sera of susceptible cattle at Kafer EL-Sheikh and EL-Beheira governorates

| Localities | Population at risk | Sample size | Positive samples | Prevalence Rate % |
|---|---|---|---|---|
| Kafer EL-Sheikh | 460 | 101 | 96 | 20.87 |
| EL- Beheira | 365 | 50 | 48 | 13.15 |
| Total | 825 | 151 | 144 | 17.45 |

Table 2: Prevalence of RVF viral antibodies in sera of Contact donkeys at Kafer El-Sheikh and EL-Beheira governorates

| Localities | Population at risk | Sample size | Positive samples | Prevalence Rate % |
|---|---|---|---|---|
| Kafer EL-Sheikh | 398 | 85 | 38 | 9.54 |
| El-Beheira | 317 | 40 | 17 | 5.36 |
| Total | 715 | 125 | 55 | 7.69 |

Table 3: Prevalence of RVF viral antibodies in sera of contact domestic birds at Kafer El-Sheikh and EL-Beheira governorates

| Localities | Population at risk | Sample size | Positive samples | Prevalence Rate % |
|---|---|---|---|---|
| Kafer EL-Sheikh | 355 | 67 | 3 | 0.85 |
| EL- Beheira | 205 | 30 | 1 | 0.49 |
| Total | 560 | 97 | 4 | 0.71 |

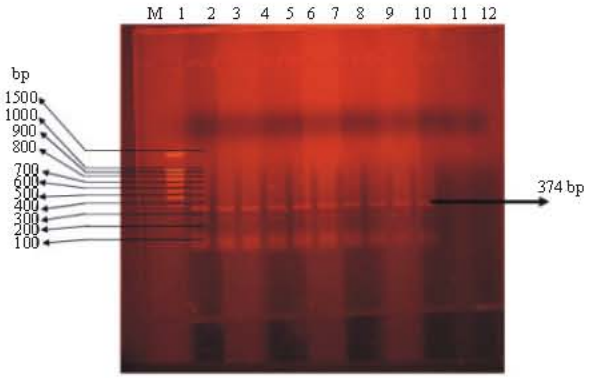


Fig. 1: RT. PCR for detection of RVF Virus in mosquitoes' pools collected during the period from Feb., 2004 to Jan., 2005 from Kafer El Sheikh governorates. M: 100 bp ladder, lanes (1: 12): each lane represents monthly collected pooles, respectively. Bold arrow indicates molecular size of positive samples.

EL-Beheira governorates) were 9.54% and 5.36, respectively. From a total of 715 donkeys at risk areas, 97 serums samples were tested against RVFv by using ELISA Results are illustrated in Table 2.

**Domestic birds:** Prevalence of RVF viral antibodies in sera of contact domestic birds at (Kafer El-Sheikh and EL-Beheira governorates) were 0.85 and 0.49%, respectively. From a Total 560 birds at risk areas, 97 serum samples were tested against RVFV by using ELISA. Results are illustrated in Table 3.

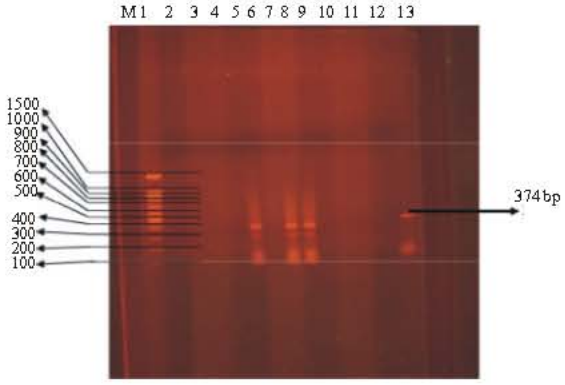


Fig. 2: RT. PCR for detection of RVF Virus in mosquitoes' pools collected during the period from Feb., 2004 to Jan., 2005 from El-Beheira governorates. M: 100 bp ladder, lanes (1: 12): each lane represents monthly collected pooles, respectively, lane 13 represents control positive. Bold arrow indicates molecular size of positive samples.

**Detection of RVFv RNA in mosquitoes:** The results obtained from this study revealed that RNA of RVFv was detected in all mosquitoes pools collected from Kafer EL-sheikh governorates except of two months (Decmber 2004 and January 2005 ). On other hand, RNA of RVFV was not detected in all mosquitoes pools collected from EL-Beheira governorates except in three months (June, Aug and Sep. 2004) (Figure 1 and 2). Positive results of the samples were obtained by present of amplified products of the expected size (374 bP).

## DISCUSSION

Egypt is an endemic area as reported by [15], in which the disease appeared then disappeared therefore vaccination is applied. This leads to the conclusion of the importance of surveillance to detect the epidemiology of the disease. So, monitoring and surveillance of the disease in hot areas such as (Kafer EL-Sheikh and EL-Beheira governorates) using different epidemiological and biotechnological tools are very necessary to investigate these points.

In this study RT-PCR and ELISA tests were conducted to determine the situation of RVF disease in Kafer EL-Sheikh and EL-Beheira governorates. ELISA appears to be a precise and technically feasible method for detecting Rift Valley Fever antibodies in Egyptian field situations [16]. It can be applied as a field screening procedure for detection of IgG class of antibodies to RVF.

[7, 16-18]. It can detect antibodies to RVFv as early as 6 days post infection. The early detection of IgG class of antibodies against RVFv which is present during infection or vaccination added more value to ELISA as a diagnostic test [7, 16]. On the other hand, PCR offers a rapid, sensitive and specific means for detecting arthropod-borne viruses [19, 20]. RT – PCR provides a promising option for diagnosis and detection of viral RNA in mosquitoes[9].

To study the prevalence of this virus, blood of susceptible animals (cattle) in infected areas, were collected for detection of RVF viral antibodies in the sera. Results showed that the prevalence rate at Kafer EL-Sheikh governorate was about 20.87%, while it was about 13.15% at EL-Beheira governorate with total prevalence rate of 17.45% during the period from (Februry 2004 to January 2005). These results agree with [21] who diagnosed the disease in cattle and sheep during 1994 in Beheira and Kafer-EL-sheikh governorates. However, these authors recorded a higher rate of prevalence (31.65%). Also, this study agrees with previous studies that revealed presence of RVFv in serum of infected cattle [22-26].

Concerning contact animals, serum samples were used to study the prevalence of RVF viral antibody in sera of donkeys and domestic birds in infected area (Kafer El- Sheikh and EL-Beheira governorates) to study the role of these contact animals as a reservoir for transmission of the RVF disease for susceptible animals by using ELISA test. Serum samples from donkeys were used for detection of viral antibodies by using ELISA test. The obtained results showed that the prevalence rate was 9.54% at Kafer EL-sheikh Governorate while it was 5.36% at EL-Beheira governorates with total prevalence rate of 7.89%. So, donkeys represent a source of infection to susceptible animals and act as amplifying host. The prevalence rate of RVF between donkeys is more prevalent in Kafer EL-Sheikh than EL-Beheira govemorates. These results agree with [22] who collected serum samples from apparently healthy donkeys in Sudan and found that precipitating antibodies of RVFv was 4% and also with [27] who pointed that blood of apparently healthy equine has a RFV during the Egyptian epizootic RVF. Moreover, it was recorded that donkeys act as amplifying host [28-30]. While, [31] demonstrated that sera of donkeys gave the lowest prevalence rate (1.5%), compared with that of previous studies. This indicates that donkeys, became a serious source for transmission of the RVF to susceptible animals.

Regarding domestic birds, serum samples were used for the detection of viral antibody by using ELISA test. The obtained results showed that the prevalence rate was 0.85% at Kafer EL–Sheikh governorate while it was 0.71% at El-Beheira governorate). This very low prevalence showed that the domestic birds may not act as a serious source for transmission of RVF disease to susceptible animals. These results agree with [6]. While disagree with Russian working on arbovirus in Guinea during 1978-1989, who reported that birds may act as a vector to RVFv. In the same time, the present study was designed to confirm the presence of RNA relevant to RVFv in mosquitoes using RT-PCR to clarify the role of mosquitoes in the transmission of the diseaser. RT. PCR was applied on a total number of 2445 mosquitoes with pools of 50 mosquitoes/ month of the study were prepared. The current results agree with [9] who detected RNA of Rift Valley Fever Virus in mosquitoes by the same technique. Primers were selected from a highly conserved region of the M. Segment to amplify a portion of the G2 glycol protein gene. RT-PCR was possible to detect a single infected mosquito in pools of 25: 50 infected mosquitoes. In addition, our results agree with [32] who detected many RVFv isolates in Egypt during epidemic of 1977 in domestic animals. On the other hand [33] recorded that no RVF virus was isolated from mosquitoes during out break in sheep in India and [17] who failed to isolate RVFv from mosquitoes during an epizootic disease. Also, [27] reported that during epizootic of RVF reappearance in Egypt (summer of 1978). El-Shinawy *et al*. [34] added that the incidence of RVF infection could be attributed to the prevalence of *Culex pippins* during summer. Moreover, [35-38] found intact relationship between summer rain fall and serological evidence of RVF.

It can be concluded that *Culex* genus play an important role as a vector in transmitting RVF virus in cattle and the spreading of the disease is more significant in Kafr El-Sheikh than El-Beheira during 2004/2005. Cattle contact animals may play an important in hibernating the disease and its recurrence by the mosquitoes.

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