



EPIDEMIOLOGICAL STUDIES ON RIFT VALLEY FEVER DISEASE IN EGYPT

Marawan A. Marawan^a, Mohamed H. Ebied^a, El-Sayed M. Galila^a, Ahmed I. Youssef^b, Karim Z. Hassan^c

^aInfectious Diseases, Department of Animal medicine, Faculty of Veterinary Medicine, Benha University,

^bDepartment of Animal Hygiene, Behavior and Zoonoses, Faculty of Veterinary Medicine, Suez Canal, University, ^cRift Valley fever department Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo

ABSTRACT

An epidemiological investigation was carried out to evaluate the current situation of Rift Valley Fever disease (RVF) in Egypt. The results of study showed that vaccinated sheep from El-Qalyubia had lower percent of antibodies against RVF with non-protective titer (1/20) while, Vaccinated sheep, goat, cattle and buffaloes from Marsa Matruh and El-Monufia revealed higher percent of antibodies against RVF with protective titer 1/40 using agar gel precipitation test (AGPT), serum neutralization test (SNT) and enzyme linked immunosorbent assay (ELISA). On the other hand non-vaccinated sheep, goats, cattle, buffaloes and camels from El-Qalyubia, El-Dakahlia, El-Sharqia and Kafr El-Sheikh exhibited different rates of antibodies against RVF among governorates in which higher rate occur in El-Sharqia followed by El-Dakahlia then Kafr El-Sheikh and lastly Qualubya. The virological analysis showed no virus isolate from sera samples of all governorates or from liver suspension of rats collected from El-Qalyubia, El-Dakahlia, El-Sharqia and Kafr el-Sheikh. Results of PCR showed that RNA of RVFV was not detected in sera samples and the cell culture in the examined animals neither from El-Sharqia and Kafr El-Sheikh governorates nor from liver suspension of rats collected from El-Qalyubia, El-Dakahlia, El-Sharqia and Kafr El-Sheikh using RT-PCR and real time PCR. On conclusion there is no circulating virus among the examined governorate under study.

KEY WORDS: AGPT, Egypt, ELISA, PCR, Rift Valley Fever disease.

(BVMJ-23 [1]: 171- 184, 2012)

1. INTRODUCTION

Rift Valley fever (RVF) is a mosquito-borne viral disease associated with large-scale epizootics/epidemics throughout Africa and the Arabian Peninsula [6, 26, 41]. RVF disease is caused by Bunyaviridae, phlebovirus, Rift Valley fever virus [7, 8, 21, 25].

Egypt suffered from several outbreaks, El-Sharqia in 1977-1978, Aswan in 1993 and finally Assiut and Aswan governorates in 1997 [2, 5, 40]. Diagnosis of RVF disease can be performed when serological tests such as CFT, ELISA, indirect immune-

fluorescence technique, and virus neutralization tests are used in combination with clinical observations and epidemiological history. ELISA is a rapid, sensitive, specific and useful tool to reveal infected animal in endemic areas or during an epizootic but the golden standard method for RVFV diagnosis is virus isolation with RT-PCR which is a specific, sensitive tool for RVF diagnosis [18, 20, 39, 43].

In Egypt, trials for controlling of RVF was done by formalin inactivated tissue culture vaccine (killed ZH-501) since 1980 [3, 13,

28]. The exact situation of the circulation of RVF virus among farm animals in Egypt is still open question. Therefore, the aim of the study is to investigate the epidemiological situation of this disease among animals in some localities in Egypt.

2. MATERIAL AND METHODS

2.1. *Animals and sampling:*

1310 blood samples were collected from sheep, goats, cattle, camels and buffaloes from six governorates (El-Qalyubia, El-Dakahlia, Sharkia, Kafr el-Sheikh, Marsa Matruh, and El-Monufia) were collected from apparent healthy animals, showing fever and others showing history of abortion in different seasons. Samples from vaccinated and non-vaccinated animals.

2.2. *Preparation of rats liver homogenates:*

Sixty rats (*R. norvegicus*) were collected from 4 governorates (El-Qalyubia, El-Dakahlia, Sharkia, Kafr el-Sheikh) either alive in metal baited traps near the edge of the farm buildings or died by shooting. Specimens from rats' organs were grounded with sterile sand in sterile mortar in maintenance media (10% suspension). Then, the specimens were centrifuged at 3000 rpm for 10 minutes. The supernatant were collected and stored at -70°C until used for virus isolation and antigen detection.

2.3. *Agar gel precipitation (AGP) test:*

It was carried out according to the method described by Eissa [10] for detection of RVF antibodies.

2.4. *Serum neutralization test (SNT):*

It was carried out according to OIE Manual [36] for detection of RVF antibodies, it was recommended that the protective titer of RVF neutralizing antibodies should be 1/40.

2.5. *Enzyme Linked Immune Sorbent Assay (ELISA):*

2.5.1. *Sandwich ELISA:*

It was used for the detection of anti-RVVFV IgG antibody in cattle sera samples and it was carried out according to Paweska *et al.* [38].

2.5.2. *Recombinant antigen based indirect ELISA for the detection of anti-RVVFV IgG antibody in sheep and goat sera:* It was carried out after Jansen *et al.* [24].

2.6. *Capture enzyme-linked immunoassay for the detection of anti-RVVFV IgM antibody in sheep, goat and cattle sera:*

It was carried out according to the method of Paweska *et al.* [38].

2.7. *Virus isolation:*

It was done on non-vaccinated animal sera samples and liver suspension of rats in tissue culture according to the manual of OIE/WHO [35].

2.8. *Total RNA extraction of inoculated cell culture, and mice tissue using TRIzol® LS Reagent Kit:* It was carried out according to the method described by Shoemaker *et al.* [45].

2.9. *PCR amplification reaction:* Thirty sera samples (10 from El-Sharqia governorate and 20 from Kafr El-Sheikh governorate) and 4 liver suspension samples of rats (one sample from each governorate) were used for conventional PCR examination according to Shoemaker *et al.* [45] using the primers enlisted in Table 1.

2.10. *Real time PCR:*

Eight pooled sera samples and inoculated cell culture from El-Sharqia and Kafr El-Sheikh governorates and one pooled liver suspension sample of rats from the mentioned governorates were subjected to real time PCR examination according Garcia *et al.* [19].

Table 1 Primers used for PCR amplification

Primer	Sequence	Length	Temp. (°C)
RVFFORI	5'- GTC TTG CTT GAA AAG GGA AAA -3'	21	55
RVFREVE	5'- CCT GAC CCA TTA GCA TG -3'	17	52

Table 2 Primers used for nested PCR

Primer	Sequence	Length	Temp. (°C)
RVFFORI	5'- GTC TTG CTT GAA AAG GGA AAA -3'	21	55
RVFREVE	5'- CCT GAC CCA TTA GCA TG -3'	17	52
RVFFORA	5'- TGCTACCAGACTCATTGTC- 3'-3'	20	52

Table 3: the reaction mixture for real time PCR

Component	Volume/reaction	Final concentration
2× QuantiTect Probe RT-PCR Master Mix	25 µl	1×
Primer S432	1µl	0.4 µM
Primer NS3m	1µl	0.4 µM
Probe CRSSAr	0.5 µl	0.2 µM
QuantiTect RT Mix	0.5 µl	
Template RNA	10 µl	
RNase-free water	12 µl	
Total reaction volume	50 µl	

3. RESULTS

3.1. Serological investigations:

3.1.1. Results of AGPT:

Sera precipitating antibodies were detected in 2.85%, 14%, 24% and 10% of sera of non-vaccinated animals at El-Qalyubia, El-Dakahlia, El-Sharqia and Kafr El-Sheikh governorates, respectively. While the percentage were 8.88%, 80% and 68% of vaccinated animals at El-Qalyubia, Marsa Matruh and El-Monufia governorates respectively

3.1.2. Results of SNT:

Sera neutralizing antibodies were detected in 4.76%, 19%, 30% and 15% of sera of non-vaccinated animals at El-Qalyubia, El-Dakahlia, El-Sharqia and Kafr El-Sheikh governorates, respectively. While the percentage was 17.77%, 94% and 80% of vaccinated animals at El-Qalyubia, Marsa Matruh and El-Monufia governorates, respectively.

3.1.3. Results of ELISA:

RVF antibodies using ELISA were detected in 4.52%, 18.5%, 29.5% and 14.5% of sera of non-vaccinated animals at El-Qalyubia, El-Dakahlia, El-Sharqia and Kafr El-Sheikh governorates respectively. While the percentage were 16.66%, 93% and 79% of vaccinated animals at El-Qalyubia, Marsa Matruh and El-Monufia governorates respectively. There is no anti RVF V IgM antibodies detected in all tested sera samples.

3.2. Virus isolation:

All sera samples from all examined animals and all governorates together with liver suspensions of rats did not show characteristic CPE against RVF on Vero cells and all appear to be negative virus isolation.

3.3. Results of RT-PCR:

All examined serum samples, inoculated cell culture of animals and liver suspension samples of rats did not showed positive bands at the target size (223bp). These results indicate that there was no viral RNA in the examined samples (Fig. 4).

Table 4 Results of AGPT tests in the six governorates

	Samples of vaccinated animals			Samples of non-vaccinated animals		
	No.	° No. of positive samples	%	No.	No. of positive samples	%
----- El-Qalyubia governorate -----						
Sheep	90	8	8.88	130	5	2.85
Goat	---	---		70	1	
Cattle	---	---		80	4	
Buffaloes	---	---		40	2	
Camels	---	---		100	0	
----- El-Dakahlia governorate -----						
Sheep	---	---		70	6	14
Goat	---	---		30	2	
Cattle	---	---		66	14	
Buffaloes	---	---		34	6	
----- El-Sharqia governorate -----						
Sheep	---	---		100	30	24
Goat	---	---		100	18	
----- Kafr El-Sheikh governorate -----						
Sheep	---	---		82	8	10
Goat	---	---		62	1	
Cattle	---	---		28	10	
Buffaloes	---	---		28	1	
----- Marsa Matruh governorate -----						
Sheep	60	44	80	---	---	---
Goat	20	18		---	---	
Cattle	20	18		---	---	
----- El-Monufia governorate -----						
Sheep	18	16	68	---	---	---
Goat	6	4		---	---	
Cattle	40	30		---	---	
Buffaloe	36	18		---	---	

° Positive samples from sera samples as it is.

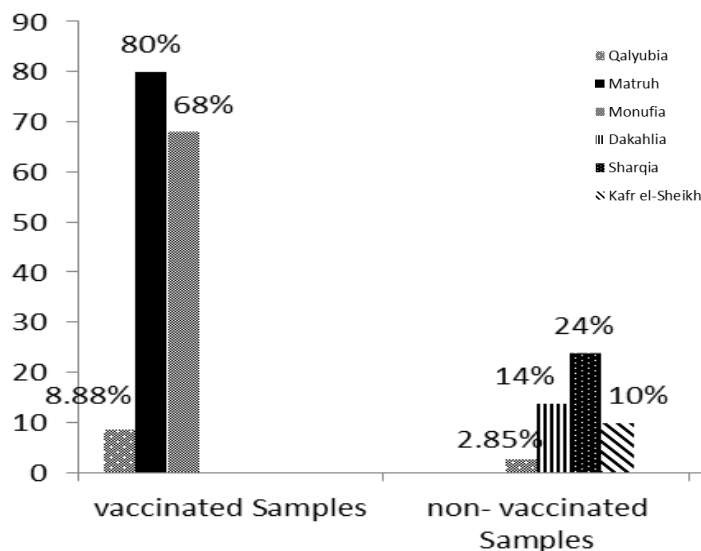


Fig. 1 Results of AGPT tests in the six governorates

Epidemiology of RVF disease in Egypt

Table 5: Results of SNT tests in the six governorates

	Samples of vaccinated animals			Samples of non-vaccinated animals		
	No.	No. of positive samples	%	No.	No. of positive samples	%
----- El-Qalyubia governorate -----						
Sheep	90	16 ●	17.77	130	7 ●	4.67
Goat	---	---		70	3 ●	
Cattle	---	---		80	6 ●	
Buffaloes	---	---		40	4 ●	
Camels	---	---		100	0 ●	
----- El-Dakahlia governorate -----						
Sheep	---	---	-----	70	10 ●	19
Goat	---	---		30	4 ●	
Cattle	---	---		66	18 ●	
Buffaloes	---	---		34	6 ●	
----- El-Sharqia governorate -----						
Sheep	---	---	-----	100	40 ●	30
Goat	---	---		100	20 ●	
----- Kafr El-Sheikh governorate -----						
Sheep	---	---	-----	82	14 ●	15
Goat	---	---		62	3 ●	
Cattle	---	---		28	12 ●	
Buffaloes	---	---		28	1 ●	
----- Marsa Matruh governorate -----						
Sheep	60	58 ○	94	---	---	---
Goat	20	18 ○		---	---	
Cattle	20	18 ○		---	---	
----- El-Monufia governorate -----						
Sheep	18	18 ○	80	---	---	---
Goat	6	5 ○		---	---	
Cattle	40	32 ○		---	---	
Buffaloes	36	25 ○		---	---	

(●) indicated Titer dilution 1/10 and (○) indicated Titer dilution 1/40

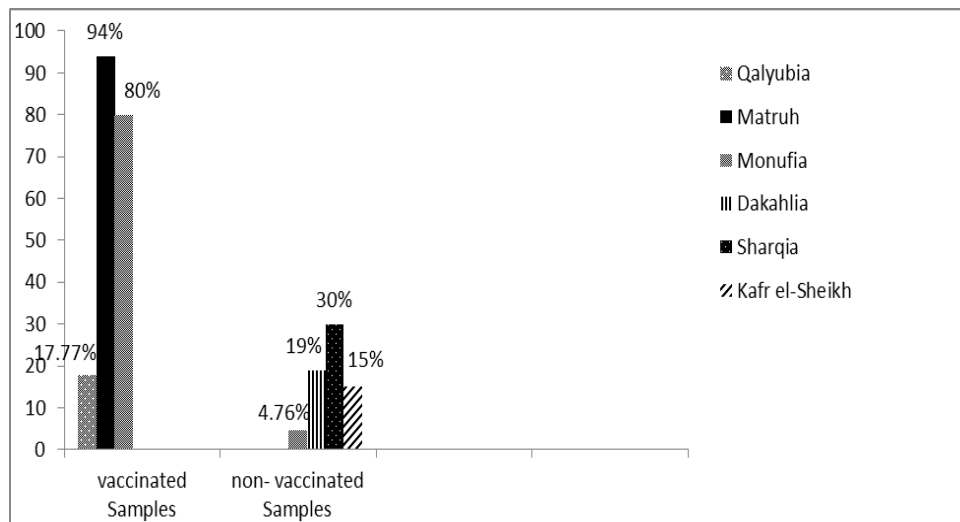


Fig. 2 Results of SNT tests in the six governorates

Table 6 Results of ELISA expresses as^o positivity percent in different governorates

Animal species	Samples of vaccinated animals			Samples of non-vaccinated animals		
	No.	No. of positive samples	%	No.	No. of positive samples	%
----- El-Qalyubia governorate -----						
Sheep	90	15		130	7	
Goat	---	---		70	3	
Cattle	---	---	16.66	80	6	4.52
Buffaloes	---	---		40	3	
Camels	---	---		100	0	
----- El-Dakahlia governorate -----						
Sheep	---	---		70	10	
Goat	---	---		30	4	
Cattle	---	---		66	17	18.5
Buffaloes	---	---		34	6	
----- El-Sharqia governorate -----						
Sheep	---	---		100	40	
Goat	---	---		100	19	29.5
----- Kafr El-Sheikh governorate -----						
Sheep	---	---		82	14	
Goat	---	---		62	3	
Cattle	---	---		28	11	14.5
Buffaloes	---	---		28	1	
----- Marsa Matruh governorate -----						
Sheep	60	54		---	---	
Goat	20	19	93	---	---	
Cattle	20	20		---	---	
----- El-Monufia governorate -----						
Sheep	18	18		---	---	
Goat	6	5		---	---	
Cattle	40	32	79	---	---	
Buffaloes	36	24		---	---	

^oCut off for IgG kits: Sheep and goat positive samples positivity percent > i= 8% and for cattle > i= 15%.

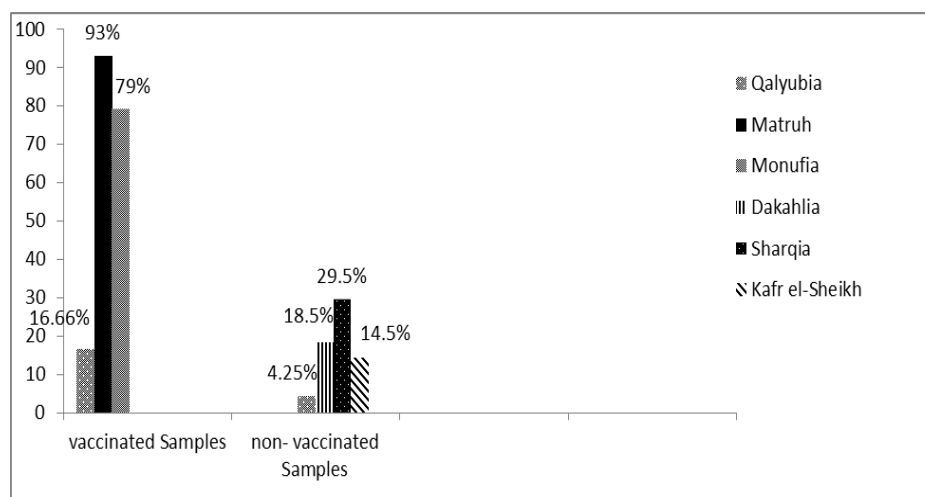


Fig. 3: Results of ELISA tests in the six governorates

3.4. Results of Real time PCR:

All examined serum samples, cell culture of animals and liver suspension samples of rats were negative and did not exceed the threshold line (CT at 19th. Cycle) after 45 cycle which indicated that there was no viral RNA in the examined samples (Fig. 5).



Fig. 4: Results of PCR on (sera samples and inoculated cell culture of animals) and liver suspension of rats. PCR Lanes: (M is 100 bp DNA ladder M), (lane C is a control positive sample with PCR amplification product sized 223 bp), while lanes 1, 2 and 3 are examined serum samples, lanes 4, 5 and 6 are examined cell culture and lanes 7, 8 and 9 are examined liver suspension samples of rats.

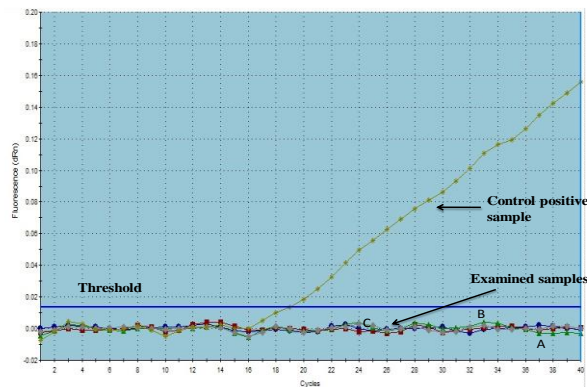


Fig. 5 Results of real time PCR on sera samples (A) and cell culture of animals (B) and liver suspension of rats (C). Plot curve of control positive samples showing threshold cycle (CT) at 19th cycle while the examined samples are negative and do not exceed the threshold line after 45 cycles.

4. DISCUSSION

Table 4 summarized the results of AGPT on serum sample collected from vaccinated animals from El-Qalyubia, Marsa Matruh and El-Monufia governorates which were 8.88%, 80% and 68% respectively. This test considers very specific but not very sensitive Abd El-Rahim *et al.*, [2], Scott *et al.* [44] and

WHO [48] in detection of RVF specific antibodies. Figure (1) showed that the positive rate of RVFV- specific antibodies vary from governorate to another in which Marsa Matruh and El-Monufia gave higher antibody positive rate than El-Qalyubia. The lower positive rate of RVFV- specific antibodies in El-Qalyubia governorate may be due to missed vaccination due to bad storage of vaccine, carelessness during vaccination or parasitic and nutritional stress. While higher positive rate of RVFV-specific antibodies in Marsa Matruh and El-Monufia governorates may be due to good and careful application of vaccine directed by Organization for Veterinary Services. The difference in positivity rate between Marsa Matruh and El-Monufia may due to differences in animal species and breeds as well as efficacy of **vaccine and vaccination. The obtained results are in agreement with results obtained by El Shinawy et al. [14] who found that Marsa Matruh governorate has the highest rate and titer of RVF specific antibodies during an epidemiological study on RVF in sheep in Egypt using AGPT.**

Table (4) summarize the results of AGPT on serum sample collected from non-vaccinated animals from El-Qalyubia, El-Dakahlia, El-Sharqia and Kafr El-Sheikh governorates which were 2.85%, 14%, 24%, 10%) respectively. Fig. 1 showed that there were positive specific antibodies against RVF with different rates among governorates in which higher rate occur in El-Sharqia followed by El-Dakahlia then Kafr El-Sheikh and lastly El-Qalyubia. The presence of these antibodies in non-vaccinated animals may be due to free animal movement among governorates or due to inter epidemic seropositivity which occur in inter epidemic period of the disease LaBeaud *et al.* [25]. These results are in agreement with those of Hubbard *et al.* [23] who reported that presence of antibodies of non-vaccinated animals at high level indicate infection either with circulating field strain or infection with

vaccinated strain (living attenuated) which produce viraemia and virus can be transmitted by mosquitoes. Our results are in disagreement with those obtained by Elian [15] who recorded high frequency of RVF antibodies in sheep sera samples in El-Qalyubia, also recorded that 7.3% of camel sera samples had antibodies. This disagreement may be due to difference in time of collection of blood samples. The results are in agreement with those of Eissa [10] who detected the presence of specific RVF antibodies in sera samples collected from non-vaccinated domestic animals using AGPT.

Table (5) and Fig. (2) summarized the results of SNT on serum sample collected from vaccinated animals from El-Qalyubia, Marsa Matruh and El-Monufia governorates which were 17.77%, 94% and 80% respectively. These results when compared with results of AGPT, indicates increase positivity percent as SNT more sensitive than AGPT [16]. The results obtained show lower antibodies titer in El-Qalyubia (1/10) which may occur due to nutritional stress on the vaccinated animals that decrease immunity of vaccinated animals to produce protective immune response (not less 1/40), while higher antibodies titer in Marsa Matruh and El-Monufia (1/40) may occur due to time of blood samples collection after vaccination (2 months) which is period of higher titer [3] and this results indicates protective titer 1/40. Eweis *et al.* [16] of anti-bodies produced in these animals which can protect against RVFV infection if occur. Our results in agreement with results obtained by Abd El-Azeim [1] who concluded that the response to vaccination program were differed with different governorates, Also obtained results are in agreement with those obtained by Nawal [32] who reported that in adult animals, the antibodies titer reach to protective level at 3rd weeks post vaccination with RVF inactivated vaccine and the animal remain protected 14-16th weeks post vaccination. The results obtained closely similar to

those of Eweis *et al.* [16] and Taha *et al.* [46] who denoted that presence of 80% specific antibodies against RVF in vaccinated sheep using SNT.

From the obtained results it was found that neutralizing antibodies against RVFV with detected in examined sera samples with history of no previous infection and history of previous vaccination against RVF, so the detected antibodies is comes from vaccination. During period of this study there was no actual outbreak occur this may be due to obligatory vaccinal program against RVF. From these results, the level of neutralizing antibodies can classify the animals into protected and non-protected according to neutralizing antibodies.

Table (5) summarized the results of SNT on serum sample collected from non-vaccinated animals from El-Qalyubia, El-Dakahlia , El-Sharqia and Kafr El-Sheikh governorates which were 4.76%, 19%, 30% and 15% respectively. Fig. (2) showed that there were positive specific antibodies against RVF with different rates among governorates in which higher rate occurs in El-Sharqia followed by El-Dakahlia then Kafr El-Sheikh and lastly El-Qalyubia. The obtained results higher than those obtained by Eissa *et al.* [12] who found that presence of RVF specific antibodies in 18.6% and 13.2% sera samples collected from non-vaccinated sheep and cattle respectively, in El-Sharqia governorate using SNT. This difference may be due to difference in season at which blood samples collected. Some results are higher and some are lower than those given by Mona [31] who found that presence 34.09% , 59.8% and 20% of RVF specific antibodies in non-vaccinated animals in North West Coast area, Aswan and North Sinai respectively, using SNT. This variation may be due to change in localities from which blood samples collected. The results indicated that percent of neutralizing antibodies against RVF in camels in El-Qalyubia governorate is 0%, this results are in disagreement with those of Eissa [11] who detected 4.7% and

6.8% in sera samples collected from slaughtered camels at Belbas abattoir and individual non-vaccinated camels that in contact with cattle herd in El-Sharqia governorate. This disagreement may be due to variation of localities and time of study as in this period (1998) outbreak appeared in Egypt or may be due to previous outbreak that occurred in this governorate.

Table (6) summarized the results of ELISA on sera samples collected from vaccinated animals from El-Qalyubia, Marsa Matruh and El-Monufia governorates which were 16.66%, 93% and 79% respectively. ELISA is a simple and safe serological test for diagnosing RVF virus in humans and animals [34]. Figure (3) showed that the results when compared with results of AGPT, indicates increase positivity percent as ELISA more sensitive and more specific 97.3% and 97.4 in detection of RVF specific antibodies [4, 17, 33, 37]. The detected antibodies by ELISA indicate IgG not IgM. In general, ELISA assays can be designed to detect either IgG or IgM antibodies that the host has produced as an immunological response to the presence of RVFV. These results are slightly higher than those of Maysa [29] who detect about 75.12% RVF specific antibodies in sera samples collected from vaccinated animals from different farms and Zagazig slaughterhouse in El-Sharqia governorates, Egypt using IgG Capture ELISA. This difference may be due to variation of localities from which blood samples collected. This variation may be due to difference in localities, age, sex and seasonal variation from which blood samples collected.

Table (6) summarized the results of ELISA on sera samples collected from non-vaccinated animals from El-Qalyubia, El-Dakahlia, El-Sharqia and Kafr El-Sheikh governorates which were 4.52%, 18.5%, 29.5% and 14.5% respectively. Fig. (3) showed that there were positive specific antibodies against RVF with different rates among governorates in which higher rate

occur in El-Sharqia followed by El-Dakahlia then Kafr El-Sheikh and lastly El-Qalyubia. The detected IgG antibodies by ELISA may attributed to either these tested animals were vaccinated in an area and moved to this localities with unknown history of vaccination or may be due to past infection. Moreover IgM did not demonstrate using IgM ELISA. The obtained results also are in agreement with results obtained by Halla [22] who investigated the sero-pervallence of RVFV antibodies in cattle, sheep, buffaloes, goats and camels sampled from Aswan, Sharkia, El-Dakahlia and Kafr El-Sheikh governorates during summer and autumn of 2009 and found that presence of specific antibodies against RVF in sera samples collected from non-vaccinated animals in these localities using IgG capture ELISA. Also the results obtained revealed that there is no significant difference between ELISA and SNT in detection of RVF specific antibodies which are in agreement with results of previous studies [2, 16, 27, 30, 44, 47] which found that ELISA was parallel to SNT in evaluation of the immune status of RVF in animal sera samples, the authors added that ELISA was found to be sensitive and accurate test. These results are slightly higher than those of Maysa [29] who detected about 20.95% RVF specific antibodies in sera samples collected from non-vaccinated animals from different farms and Zagazig slaughterhouse in El-Sharqia governorates, Egypt using IgG Capture ELISA. This difference may be due to variation of seasons at which blood samples were collected.

The results of virus isolation from non-vaccinated animal sera samples on tissue culture revealed that there is no circulating virus in the collected sera samples from the six governorates during this study. These results are in disagreement with the results obtained by Zaghawa *et al.* [49] who reported that the virus was isolated from 29.5% and 57.1% in Behera and Kafr El-Sheikh governorates respectively,

during an epidemiological studies on RVF in Egypt during 1994. This disagreement may be due to presence of clinical signs of RVF in the tested animals or also may be due to the outbreak that occurred in 1993 in Aswan governorate with spreading of virus by insect.

According to our knowledge, there is no published report till now in using RT-PCR and real time PCR on sera samples of animals in diagnosis of RVF in Egypt.

Figure (4) showed that no positive bands at the target size (223bp), this indicates that there is no viral RNA in the examined sera samples and cell culture collected from El-Sharqia and Kafr El-Sheikh governorates which means no virus was present. Figure (5) showed that sera samples and cell culture are completely negative and not exceed the threshold line (CT at 19th. Cycle) after 45 cycle which indicates that there is no viral RNA in the examined samples. RT-PCR and real time PCR are very sensitive and very specific in detection of RVF viral RNA in sera samples and cell culture collected from animals and human [42]. The obtained results are in disagreement with those of Reham *et al.* [42] who proved direct detection of RVFV infection in sera samples and cell filtrate of animals in Sudan using RT-PCR. This disagreement may occur as Sudan contains RVF since the last outbreak in 2007.

Results obtained of virus isolation from liver suspension of rats on tissue culture revealed that there is no characteristic CPE of RVF so no circulating virus in the collected sera samples from El-Qalyubia, El-Dakahlia, El-Sharqia and Kafr El-Sheikh governorates during the study.

Figure (4) show no positive bands at the target size (223bp), this indicates that there is no viral RNA in the examined liver suspension samples of rats collected from El-Qalyubia, El-Dakahlia, El-Sharqia and Kafr El-Sheikh governorates which means no virus present. Figure (5) showed that liver suspension samples are completely negative and not exceed the threshold line

(CT at 19th Cycle) after 45 cycle which indicates that there is no viral RNA in the examined samples. The obtained results are also in disagreement with those of Mona [31] who detected that presence of 3.3% and 5% positive RVF viral RNA in liver suspension of rats collected from North Sinai and North West Coast area respectively, using RT-PCR. This disagreement may be due to that collected samples were during or after short period of incidence of outbreak.

Finally we concluded that the highest incidence of antibodies recorded in the present study in non-vaccinated animals, indicated that the virus may still circulating in the presence of insect vector. Regular vaccination as well as insect control by different means should be followed, to minimize the number of susceptible animals as well as neutralizing the role of insect to both mechanical and biological transmission.

On conclusion: the results revealed presence of circulating RVFV among susceptible animal species in the governorate under study.

5. REFERENCES

1. Abd El-Azeim, F.M. 2004. Some studies on Rift valley fever in El-Dakahlia and Dumiat Governorates PhD, Fac. Vet. Med., Zagazig University.
2. Abd El-Rahim, I.H.; Abd El-Hakim, U. and Hussein, M. 1999. An epizootic of Rift Valley fever in Egypt in 1997. *Rev Sci Tech* **18**: 741-748.
3. Abdel-Ghaffar, S., El-Nimr, M.M., Mohsen, A.Y. El-Nakashly, S., Attia, M. and Emad, N. 1981. Seroconversion of sheep vaccinated with inactivated RVF vaccine. *Bull. De L'office Int. Des Epizootic.* **39**: 1379-1385.
4. Abouzaid, A.A., Nakashly, S.A., Nasr, M.Y., Mokhbatly, A.A. and Khafagy, A.A. 1995. Studies on Rift Valley Fever in sheep. *J. Egypt. Vet. Med. Ass.* **55**: 339-351.
5. Arthur, R.R., El-Sharkawy, M.S., Cope, S.E., Botros, B.A., Oun, S., Morrill, J.C.,

- Shope, R.E., Hibbs, R.G., Darwish, M.A. and Imam, I.Z. 1993. Recurrence of Rift Valley fever in Egypt. *Lancet* **342**: 1149-1150.
6. Bird, B.H., Khristova, M.L., Rollin, P.E., Ksiazek, T.G. and Nichol, S.T. 2007. Complete genome analysis of 33 ecologically and biologically diverse Rift Valley Fever virus strains reveals widespread virus movement and low genetic diversity due to recent common ancestry. *J. Virol.* **81**: 2805–2816.
 7. Chevalier, V., Pepin, M., Plee, L., Lancelot, R. 2010. Rift Valley fever a threat for Europe. *Euro Surveill.* **15** : 19506 (<http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19506>)
 8. Daubney, R., Hudson, J.R. and Garnham, P.C. 1931. Enzootic hepatitis or Rift Valley fever. An undescribed virus disease of sheep, cattle and man from East Africa. *J. Path. Bacter.* **34**: 545-579.
 9. Davies, F.G. 1975. Observations on the epidemiology of Rift Valley Fever in Kenya. *J. Hygiene* **75**: 219 – 230.
 10. Eissa, M.I. 1984. Preliminary survey of domestic animals of the Sudan for precipitating antibodies to Rift Valley fever virus. *J. Hyg. Camb.* **93**, 629-637.
 11. Eissa, M.I. 1998. Serological survey against some viral diseases in camels in El-Sharqia governorate, Egypt. Proceedings of the Third Annual Meeting for Animal Production under Arid Conditions 1. Pp. 167-173.
 12. Eissa, M.I., Abou Zaid, A.A. and Ahmad, M.H. 1999. Studies on Rift Valley fever epizootic and role of ticks (*hyalomma* spp) in virus transmission. *J. Egypt. Vet. Med. Assoc.* **59**: 1585-1596.
 13. El Nimr, M.M., Abdel Gaffar, S., Mohsen, A.Y., El Debegy, A., El Danaf, N.A., El Nakashly, S., Mohamed, Z. and Emad Nafie 1981. Infection of BHK cells with Rift Valley Fever V. *Bull of Int. Epiz.* **93**: 1351 - 1359.
 14. El Shinawy, M., Hussein, N.A., El-Nimr, M.M., Mohsen, A.Y., Zaghawa, A.A., Wassel, M., El-Ebiary, E. and Zaki, F. 1993. An epidemiological study of Rift Valley Fever in sheep in Egypt. *Assiut Vet. Med. J.* **28**: 128-139.
 15. Elian, K.A. 1983. Detection of complement fixing and hemagglutinating inhibiting antibodies against Rift Valley Fever virus in sera of domestic animals in Qalyubia Governorate. M.V.Sc., Fac. Vet. Med., Alex. Univ., Egypt.
 16. Eweis, M., Samya, S. Abdel-Naby and Saber, M.S. 2008. Comparative studies on the different laboratory diagnostic methods for Rift Valley Fever virus in domestic animals Egypt. *J. Comp. Path. & Clinic. Path.* **21**: 136-147.
 17. Fafetine, J.M., Tijhaar, E., Paweska, J.T., Neves, L.C., Hendriks, J., Swanepoel, R., Coetzer, J.A., Egberink, H.F. and Rutten, V.P. 2007. Cloning and expression of Rift Valley fever virus nucleocapsid (N) protein and evaluation of a N-protein based indirect ELISA for the detection of specific IgG and IgM antibodies in domestic ruminants. *Vet. Microbiol.* **121**: 29-38.
 18. Flick, R. and Bouloy, M. 2005. Rift Valley fever virus. *Curr. Mol. Med.* **5**: 827-834.
 19. Garcia, S., Crance, J.M., Billecocq, A., Peinnequin, A, Jouan, A, Bouloy, M, and Garin, D. 2001. Quantitative Real-Time PCR Detection of Rift Valley Fever virus and its application to evaluation of antiviral compounds. *J. Clin. Microbiol.* **39**: 4456-4461.
 20. Gerdes, G.H. 2004. Rift Valley Fever, in: Office International des Epizooties (Ed.), Manual of diagnostic yeasts and vaccines for terrestrial animals (mammals, birds and bees), Chapter 2.1.8.; Paris. Pp. 185–194.
 21. Gubler, D.J. 2002. The global emergence/resurgence of arboviral diseases as public health problems. *Arch. Med. Res.* **33**: 330–342.
 22. Halla, E.K. 2011. Effect of Environmental and Ecological Factors on the Epidemiology of Rift Valley fever disease. M.V.Sc., Fac.Vet. Med, Benha University.
 23. Hubbard, K.A., Baskerville, A., Stephenson, J.R. 1991. Ability of a mutagenized virus variant to protect young lambs from RVF. *Am. J. Vet. Res.* **52**: 50-55.
 24. Jansen van Vuren, P., Potgieter, A.C., Paweska, J.T. and Van Dijk, A.A. 2007. Preparation and evaluation of a recombinant Rift Valley fever virus N protein for the detection of IgG and IgM antibodies in humans and animals by indirect ELISA. *J. Virol. Meth.* **140**: 106-114.

25. LaBeaud, A.D., Muchiri, E.M., Ndzovu, M., Mwanje, M.T., Muiruri S., Muiruri, C.J.M. and King, C.H. 2008. Interepidemic Rift Valley Fever virus seropositivity, Northeastern Kenya. *Emerg Infect. Dis.* **14**: 1240-1246.
26. Laughlin, L.W., Meegan, J.M., Strausbaugh, L.W., Morens, D.M. and Watten, R.H. 1979. Epidemic Rift Valley fever in Egypt: observations of the spectrum of human illness. *Trans. R. Soc. Trop. Med. Hyg.* **73**: 630-633.
27. Lily, S.S., Eman, M.S., Marcoss, T.N. and Gihan, K.M. 1999. Preliminary studies for rising the immune response in cattle vaccinated with inactivated RVF vaccine using zinc and copper. *Vet. Med. J. Giza* **47**: 389-396.
28. Marcoss, T.N. 1992. Attempts of preparing a combined RVF vaccine with sheep pox vaccine for sheep in Egypt. M.V.Sci., Fac. Vet. Med., Alexandria University, Egypt.
29. Maysa, A.E. 2006. Some epidemiological studies on Rift Valley fever. Ph.D., Fac. Vet. Med., Zagazig University.
30. Meegan, J.M., Yedloutsching, R.J., Peleg, B.A., Shy, J., Peters, G.L., Walker, J.S. and Shope, R.E. 1987. ELISA for detection of antibodies to RVF virus in ovine and bovine sera. *Am. J. Vet. Res.* **48**: 1138-1141.
31. Mona, A.M. 2000. Studies on Rift Valley Fever in border governorates. Ph.D., Fac. Vet. Med., Cairo Univ.
32. Nawal, M.A.Y. 1984. The antibodies response of pregnant ewes to RVFV vaccine and the resulting maternal immunity. M.V.Sc., Fac. Vet. Med, Cairo University.
33. Niklasson, B., Liljestrand, J., Bergstr, M.S. and Peters, C.J. 1987. Rift Valley fever: a sero-epidemiological survey among pregnant women in Mozambique. *Epidemiol. Infect.* **99**: 517-522.
34. Niklasson, B., Peters, C.J., Grandien, M. and Wood, O. 1984. Detection of human IgG and IgM antibodies to Rift Valley Fever V by ELISA. *J. Clin. Micro.* **19**: 225-229.
35. OIE / WHO 2005. Manual of Diagnostic tests and vaccines for terrestrial Animals. (www.oie.int.)
36. OIE Manual 1996. Manual for standards for diagnostic tests and vaccines.
37. Paweska, J.T., Barnard, B.J.H. and Williams, R. 1995. The use of sucrose-acetone-extracted Rift Valley fever virus antigen from cell culture in an indirect enzyme-linked immunosorbent assay and haemagglutination-inhibition tests. *Onderstepoort. J. Vet. Res.* **62**. 227-233.
38. Paweska, J.T., Burt, F.B., Anthony, F., Smith, S.J., Grobbelaar, A.A., Croft, J.E., Ksiazek, T.G., Swanepoel, R. 2003. Ig-G sandwich and IgM-capture enzyme-linked immunosorbent assay for the detection of antibody to Rift Valley fever virus in domestic ruminants. *J. Virol. Meth.* **113**: 103-112.
39. Paweska, J. T., Mortimer, E., Leman, P. A., Swanepoel, R. 2005. An inhibition enzyme-linked immunosorbent assay for the detection of antibody to Rift Valley fever virus in humans, domestic and wild ruminants, *J. Virol. Meth.* .127:10–18.
40. Paweska, J.T., Van Vuren, P.J., Kemp A., Buss, P., Bengis, R.G. and Gakuya, F. 2008. Recombinant nucleocapsid based ELISA for detection of IgG antibody to Rift Valley fever virus in African buffalo. *Vet. Microbiol.* **127**: 21-28.
41. Peters, C.J. 1997. Emergence of Rift Valley fever. In: Factors in the emergence of *Arbovirus* disease. Saluzzo, J.F., Dodet B, (eds.). Paris: Elsevier. Pp. 253–63.
42. Reham, W.S, Khairalla, M.S, Awadalkareem, A.E, Abdelrahim, E.K. and Imadeldn, E.A. 2010. A single tube RT-PCR amplification for detection of Rift Valley fever virus. *Res. J. of med. Sci.* **4**: 149-151.
43. Sall, A.A, Macondo, E.A, Sène, O.K., Diagn, M., Sylla, R., Mondo, M., Girault, L., Marrama, L., Spiege, A., Diallo, M., Bouloy, M. and Mathiot, C. 2002. Use of Reverse Transcriptase PCR in Early Diagnosis of Rift Valley Fever. *Clin. Diag. Lab. Immunol.* **9**: 713–715.
44. Scott, R.M., Feinsod, F.M., Allam, I.H., Ksiazek, T.G., Peters, C.J., Botros, B.A. and Darwish, M.A. 1986. Serological tests for detecting Rift Valley fever viral antibodies in sheep from the Nile Delta. *J. Clin. Microbiol.* **24**: 612-614.
45. Shoemaker, T., Boulianne, C., Vincent, M.J., Pezzanite, L., Al-Qahtani, M.M., Khan, Y.A., Ali, S., Rollin, P.E., Swanepoel, R., Ksiazek, T.G., and Nichol, S.T. 2002. Genetic analysis of viruses

- associated with emergence of Rift Valley Fever in Saudi Arabia and Yemen. *Emerg. Infect. Dis.* **8**: 1415–1420.
46. Taha, M.M., Khirate, E.R., Elian K., Gihan, K.M., Soad, M.S., Mohsen, A.Y. and Aida EL Debegy 1994. Studies on RVFV. Trials for production of attenuated RVF variant from original (ZH501) Egyptian strain. *J. Egypt. Vet. Med. Assoc.* **54**. Cited in: Comparative studies on the different laboratory diagnostic methods for Rift Valley Fever virus in domestic animals. By: Eweis, M., Samya S. Abdel-Naby and Saber, M.S. 2008. *Egypt. J. Comp. Path. Clinic. Path.* **21**: 136 - 147
47. Taha, M., Khirate Elian., Gihan, K., Hassan, K. and Fatma, S. 2002. Standardization of both Rift Valley Fever cell lysate and supernatant antigens used in ELISA comparing with SNT and AGPT. *Vet. Med. J. Giza* **50**: 987-992.
48. World Health Organization 1982. "RVF" Laboratory diagnostic procedures. Work shop on RVF held at Aristotation Univ. of Thessalonik, Greece, Sponord by WHO, UNDP.
49. Zaghawa, A. and Kheirat, E. 1995. Some epidemiological aspects on Rift Valley fever in Egypt during 1994. 3rd Sci. Cong., Egyptian Society for cattle diseases, Dec, Assiut, Egypt. Pp. 3-5.



دراسات الوبائية عن مرض حمى الوادي المتصدع في مصر

مروان عادل مروان¹، محمد حسنين عبيد¹، السيد مصطفى جليلة¹، احمد ابراهيم يوسف²، كريم زكي حسن³

¹قسم طب الحيوان، كلية الطب البيطري، جامعة بنها، ²قسم طب الامراض المشتركة وصحة وسلوك الحيوان، كلية الطب البيطري، جامعة قناة السويس، ³وحدة الحمى القلاعية، معهد بحوث الامصال واللقاحات البيطرية بالعباسية، القاهرة

الملخص العربي

تم إجراء دراسة وبائية علي وجود فيروس مرض حمى الوادي المتصدع في مصر في الفتره الحاليه. الأغنام المحصنه (المجمعة من القليوبيه) اظهرت وجود اجسام مناعيه لحمى الوادى المتصدع بمعدل قليل وتحت حدود مستوى المناعة 20/1 والاغنام , الماعز , الماشيه والجاموس والجمال المحصنه (المجمعة من مرسى مطروح والمنوفيه) اظهرت وجود أجسام مناعية ضد المرض بمعدل عالي وفي حدود مستوى المناعة 40/1 باستخدام اختبار الترسيب واختبار السيرم التعادلي واختبار الاليزا. اما الاغنام, الماعز, الماشيه , الجاموس والجمال الغير محصنة (المجمعه من القليوبيه والدقهليه والشرقيه وكفرالشيخ) أظهرت وجود اجسام مناعيه ضد المرض بمعدلات مختلفه بين المحافظات حيث وجدت بكميه عاليه في الشرقيه ثم الدقهليه و كفر الشيخ واخيرا القليوبيه وذلك بعد الاختبارات المخبريه الثلاث كما أظهرت النتائج عدم عزل الفيروس من عينات السيرم ولا عينات خلايا السيرم المجمعه من الست محافظات وكذلك من انسجه كبد الفئران المجمعه من القليوبيه والدقهليه والشرقيه وكفرالشيخ وذلك علي خلايا الزرع النسيجي (فيرو). كما لم يتم تحديد وجود الحامض النووي للفيروس من عينات السيرم او عينات خلايا السيرم المجمعه من الشرقيه وكفر الشيخ ومن انسجه كبد الفئران المجمعه من القليوبيه والدقهليه والشرقيه وكفرالشيخ باستخدام RT-PCR و real time PCR. خلاصة الدراسه: ان المعطيات الوبائيه التي تم الحصول عليها اثبتت عدم وجود فيروس ساري او وجوده في صورته خفيه لا ترقى الي صورته الاكلينيكيه. (مجلة بنها للعلوم الطبية البيطرية: عدد 23 (1)، يونيو 2012: 171-184)