Epizootological Study and Molecular Diagnosis on Rift Valley Fever Disease In The Egyptian Border Governorates

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Abstract:

Rift valley fever (RVF) is a zoonotic disease which affects both humans and animals. It is caused by a Arbovirus which is maintained in nature through transmission between susceptible vertebrate hosts and blood sucking arthropods such as mosquitoes. In this study, an epidemiological investigation was carried out to evaluate the current situation in nine border Governorates of Egypt for Rift Valley Fever disease (RVF). Nine hundred thirty serum samples were collected randomly from Aswan, Red Sea, Alwadi El-Gdid, Marsa Matroh, North Sini, South Sini, Seuze, Ismailia and Port Said governorates. Samples were collected from different animal species such as cattle, camel, sheep and goat from May to October 2013. Serodiagnostic studies were done on serum samples by using commercial competitive ELISA for detection of IgM and IgG antibodies against Rift valley fever virus. 64 out of 930 samples in percentage of 9.3% were positive for detection of IgG antibodies against RVFV and all serum samples were negative for detection of IgM against RVFV. To confirm that the presence or absence of RVFV in the positive serum samples, RT-PCR using primers targeting the S segment was carried out. Sixty four positive serum samples and collected insects were subjected to rt-PCR to amplify 390 bp S segment of RVFV and the results were confirmed negative for all.

Key words: Rift Valley Fever Disease, ELISA, RT-PCR

1- Introduction

Rift Valley fever (RVF) is a vector-borne, zoonotic disease characterized by abortion storms which increases serious morbidity and mortality in both humans and livestock. The disease is caused by the RVF virus which belongs to the genus Phlebovirus, in the family Bunyaviridae. RVF mainly affects domestic animals such as cattle, goats, sheep and camels, as well as humans **(Meegan and Bailey, 1989).** The RVF virus is present in most African countries and the Middle East (Saudi Arabia and Yemen). The emergence of this disease has been steady, expanding out of Sub-Saharan Africa and Egypt in 1977 **(Swanepoel and Coetzer, 2004).** The distribution of RVFV has been well documented in many African countries, particularly in the north (Egypt, Sudan), east (Kenya, Tanzania, and Somalia), west (Senegal, Mauritania) and south (South Africa), but also in the Indian Ocean (Madagascar, Mayotte) and the Arabian Peninsula **(Pourrut *et al.*, 2010).**

In recent years, the virus has been particularly active in Mauritania, there were outbreaks in 2010, 2012 and 2015 as well as in Senegal there were outbreaks in 2013 and 2014. Recent studies from 2008 and 2014 conducted in the Maghreb countries indicated that the RVF virus could be present in certain regions of Algeria, Morocco and Tunisia. **(Bosworth et al., 2016).** In Egypt RVFV was first introduced in July, 1977 **(Laughlin *et al.*, 1979)**. An outbreak of RVF began in the Aswan Province, and then spread up the Nile Valley, Sharqiya, Qalyobia, Giza, Sohag, Assiut and Minya. The 1977 RVF outbreak in Egypt resulted in an estimated 18,000 infections and 598 deaths in humans. **(Ali and Kamel 1978; Meegan, 1979).** In late May, 1993, RVF disease had occurred in Aswan province in Egypt infecting both humans and domestic animals after 12-years of absence with unknown source of re-introduction **(Arthur *et al.*, 1993).** Another outbreak had occurred in Aswan and Assuit Provinces, between April and August 1997. The importation of infected ruminants, especially camels from Sudan was the principle source of infection. Aswan is the nearest Egyptian province to Sudan which considered the focus of RVFV infection in Egypt **(Abd Elrahim *et al.*, 1999).** Forty-five

cases of RFV including 17 deaths in Seedy Salem district were recorded and all cases were recorded from Egyptian farmers **(WHO, 2003)**.

2- Material and Methods

1- Serum samples

Random serum samples were collected from different species without animal ID and No case history of previous vaccination for all animals under study. A total 930 serum samples were collected from animals of different species, governorates (District& Villages), age and sex (Cattle, Sheep, Goat and Camel) in the borders of Egyptian governorates (Red Sea, Marsa Matroh, Alwadi Elgdid, North Sini, South Sini, Seuze, Ismailia, Port Said and Aswan) during the period from May /2013 to October /2013.The samples were transported immediately on ice packed thermos and stored at -20°C until used.

Animal species	**Cattle**	**Sheep**	**Goats**	**Camel**
Number of serum samples	**259**	**256**	**173**	**242**

2- Insects (Mosquitoes)

2-1 Collection sites

The sites at which mosquitoes have been collected are these are basically defined in terms of farm premises, slaughtering houses and their vegetation. Zone1 in Darawa (3 traps), zone2 in Nasr lake (3 traps), zone 3 in Abu-Snible (10 traps)

2-2 Collection of mosquito samples: A total of 872 mosquitoes (50 mosquito groups) like Culex (33 pools), Aedes (15 pools) and Anopheles (2 pools) were collected with cooperation with medical institute of insects and animal health institute, Mosquitoes were collected from area positioned at ground level near (slaughter houses, quarantines area of camel and from the same places where animal blood samples were obtained according to **(Gad et al. 1995).** The collecting started from May to October 2013 by means of battery powered miniature light trap. Mosquitoes were identified according to **(Maysa, 2006 and Youssef et al, 2008).** Pools of 50 mosquitoes

were prepared for molecular diagnosis of RVFv by using rt. PCR.

2-3-ELISA Kits

2-3-1 Rift valley fever IgM capture ELISA To detect IgM antibodies directed against the Rift valley fever (RVF) nucleoprotein (NP) in bovine, ovine and caprine serum. The presence of IgM antibodies in the serum sample helped indicate recent infection. Test procedure according to ID vet Innovative Diagnostics Kits - France

2-3-2 Rift valley fever competition multi species ELISA to detect (**IgM/IgG)** antibodies direct against the RVFV nucleoprotein (NP) in serum or plasma. The detection of anti-nucleoprotein antibodies indicates exposure to the virus by natural infection or by vaccination. Test procedure according to ID vet Innovative Diagnostics Kits - France

2-4 Purification of RNA according to the GeneJET™ (thermo scientific geneJET RNA purification kit #K0731, #K0732) for real time PCR **(Sall et al., 2001)**

2-4-1 Application of rt-PCR on RNA samples: Primers used in PCR

Amplified were performed using QiaAmp viral RNA Mini kit (Qingen) according to the manufacture instruction **(Drosten et al,. 2002),** Primers were diluted in TE buffer to a working solution (20 pmol) and aliquoted and stored at -20 ºC.

3- Results

Table (1). Results of ELISA test for detection of RVFv antibodies (IgM) collected from all species all over governorates

Governorates	**District**	**Total**	**Species**							
			Cattle		**Camel**		**Sheep**		**Goats**	
			+ve	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**
Aswan	Aswan	**80**	-	32	-	10	-	21	-	17
	Draw	**56**	-	13	-	32	-	3	-	8
	Edfo	**101**	-	43	-	-	-	33	-	25
	Nase El-Noba	**50**	-	23	-	-	-	15	-	12
	Komobo	**61**	-	26	-	-	-	25	-	10

	Elsbayea	**21**	-	9	-	-	-	6	-	6
	Abu-Smbl	**200**	-		-	200	-	-	-	-
Red Sea	Halayb	**37**	-	8	-	-	-	18	-	11
	Shlatin	**23**	-	2	-	-	-	12	-	9
Alwadi El-Gdid	Elkharga	**16**	-	9	-	-	-	7	-	-
	Elfrafra	**23**	-	8	-	-	-	10	-	5
	Blat	**21**	-	8	-	-	-	8	-	5
Marsa Matroh	Matroh	**12**	-	3	-	-	-	6	-	3
	Elhamam	**9**	-	3	-	-	-	4	-	2
	El-Dbaa	**10**	-	2	-	-	-	5	-	3
	Sewa	**10**	-	4	-	-	-	3	-	3
	Ras Elhekma	**13**	-	3	-	-	-	6	-	4
	Sedi Brane	**9**	-	3	-	-	-	4	-	2
	Elsaloom	**7**	-	2	-	-	-	2	-	3
North Sini	Peer El-Abd	**19**	-	24	-	-	-	53	-	32
	Rafh	**10**	-	3	-	-	-	2	-	5
	El-Hasnh	**26**	-	35	-	-	-	64	-	35
	Nakhl	**17**	-	13	-	-	-	35	-	23
	El-Kseema	**9**	-	3	-	-	-	2	-	4
	Awal El-Aresh	**19**	-	42	-	-	-	62	-	23
South Sini	Abo-Redees	**20**	-	32	-	-	-	46	-	32
Seuze	Qesm El-Arbaeen	**10**	-	5	-	-	-	5	-	-
Ismailia	El-Tal El-Kbeer	**11**	-	5	-	-	-	4	-	2
	Elaksaseen	**11**	-	7	-	-	-	2	-	2
	Ismalia	**8**	-	3	-	-	-	4	-	1

Port Said	Elaboty	**10**	-	2	-	-	-	5	-	3
Total (930)	**(31)district**	**930**	**-**	**259**	**-**	**242**	**-**	**256**	**-**	**173**

All samples were negatives for the detection of anti-rift valley fever nucleoprotein (NP) IgM antibodies. 930/930 (100%) negative.

Table . 2. Results of ELISA test for detection of RVFv antibodies (IgG/IgM) collected from all species all over governorates

Governorate	**District**		**Number Of Animal Samples**							
	City	**Village**	**Cattle**		**Camel**		**Sheep**		**Goats**	
			+ve	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**	**+ve**	**-ve**
Aswan	Aswan	32	-	32	-	10	5	16	5	12
	Draw	14	-	13	-	32	-	3	-	8
	Edfo	44	10	34	-	-	3	30	-	25
	Nase El-Noba	24	-	23	-	-	-	15	-	12
	Komobo	29	-	26	-	-	-	25	-	10
	Elsbayea	12	-	9	-	-	-	6	-	6
	Abu-Smbl	quarantine	-		41	159	-	-	-	-
Red Sea	Halayb	5	-	8	-	-	-	18	-	11
	Shlatin	1	-	2	-	-	-	12	-	9
Alwadi El-Gdid	Elkharga	Abdel-slam aarf	-	9	-	-	-	7	-	-
	Elfrafra	Othman	-	8	-	-	-	10	-	5
	Blat	domarya	-	8	-	-	-	8	-	5
Marsa Matroh	Matroh	El-zoayrat	-	3	-	-	-	6	-	3
	Elhamam	El-shmama	-	3	-	-	-	4	-	2
	El-Dbaa	El-zyton	-	2	-	-	-	5	-	3
	Sewa	Sewa	-	4	-	-	-	3	-	3
	Ras Elhekma	Al-hekma	-	3	-	-	-	6	-	4
	Sedi Brane	Al-omda	-	3	-	-	-	4	-	2

	Elsaloom	El-salom	-	2	-	-	-	2	-	3
North Sini	Peer El-Abd	Al-khrba Abo-sadan	-	2 4	-	-	-	5 3	-	3 2
	Rafh	Rafh	-	3	-	-	-	2	-	5
	El-Hasnh	Gefgatah El-gady	-	3 5	-	-	-	6 4	-	3 5
	Nakhl	El-brok El-tamd	-	1 3	-	-	-	3 5	-	2 3
	El-Kseema	El-makdia	-	3	-	-	-	2	-	4
	Awal El-Aresh	El-skaska El-tweel	-	4 2	-	-	-	6 2	-	2 3
South Sini	Abo-Redees	-Aboznema -tgmaat abordees	-	3 2	-	-	-	4 6	-	3 2
Seuze	Qesm El-Arbaeen	El-arbaeen	-	5	-	-	-	5	-	-
Ismailia	El-Tal Elkbeer	El-balwa	-	5	-	-	-	4	-	2
	Elaksaseen	El-marzokea	-	7	-	-	-	2	-	2
	Ismalia	El-warsha	-	3	-	-	-	4	-	1
Port Said	Elaboty	Elaboty el-gdid	-	2	-	-	-	5	-	3
Total (930)	**31**	**188+ quarantine**	**10**	**249**	**41**	**201**	**8**	**248**	**5**	**168**
			259		**242**		**256**		**173**	

Among the 930 collected serum samples from various animal species (camels, cattle, sheep and goats) the governorates borders were assessed by ID vet screen® France RVF competition multispecies ELISA kits. 64 (9.3%) samples were found positive and carried antibodies (IgG/IgM) against the RVF nucleoprotein.

Table. 3. Results of ELISA test for detection of RVFv antibodies (IgM and IgG) collected from cattle in 9 governorates **in relation to age, sex, season and housing.**

Localities	**Cattle**													
	Sex				**Age**									
	male		**Female**		**6m-1y**		**1-2y**		**2-5y**		**Up to5y**		**Total**	
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Aswan	-	2	-	30	-	6	-	10	-	16	-	-	-	32
Draw	-	1	-	12	-	3	-	3	-	7	-		-	13
Edfo	-	5	**10**	29	**2**	5	**2**	10	**5**	18	**1**	1	**10**	34
Nase El-Noba	-	2	-	21	-	10	-	10	-	3	-	-	-	23
Komobo	-	3	-	23	-	6	-	10	-	10	-	-	-	26
Elsbayea	-	-	-	9	-		-	4	-	5	-	-	-	9
Abu-Smbl	-	-	-	-	-	-	-	-	-	-	-	-	-	
Red Sea	-			10				5		5	-	-	-	10
Alwadi El-Gdid	-	**6**	-	19	-	5	-	10	-	8	-	2	-	25
Marsa Matroh	-	3	-	17	-	3	-	7	-	9	-	1	-	20
North Sini	-	4	-	26	-	5	-	9	-	14	-	2	-	30
South Sini	-			5	-	-	-	1	-	4	-	-	-	5
Seuze	-	-	-	5	-	-	-	1	-	4	-	-	-	5
Ismailia	-	-	-	15	-	-	-	5	-	10	-	-	-	15
Port said	-	-	-	2	-	-	-	2	-		-	-	-	2
Total(259)	**-**	**26**	**10**	**223**	**2**	**43**	**2**	**87**	**5**	**113**	**1**	**5**	**10**	**249**

Ten out of 259 cattle samples were positive for RVF antibodies by IDvet screen® France RVF competition multispecies ELISA kits. These samples were obtained from 10 (3.9%) female cattle of different ages from the Edfo district with no cases of previous vaccination for all animals under study.

Table. 4. Results of ELISA test for detection of RVFv antibodies (IgM and IgG) collected from camel in Aswan governorate **in relation to age, sex, season and housing.**

Localities	Camel													
	sex				Age									
	male		female		6m-1y		1-2y		2-5y		Up to5y		total	
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Aswan		8	-	2	-	-	-	2	-	8	-	-		10
Draw	**19**	-	-	13	-		**5**	-	**14**	8	-	5	**19**	13
Abu-smbl	**22**	178							**22**	135	-	43	**22**	178
Total(242)	**(41)**	186	-	15	-	-	**5**	2	**36**	151	-	48	**41**	**201**

Forty one out of 242 camel samples were positive for RVF antibodies by ID screen® RVF competition multispecies ELISA kits. These samples 41 (16.9%) were obtained from males of different ages at darw and Abu-smble with no case history of previous vaccination for all animals under study.

Table. 5. Results of ELISA test for detection of RVFv antibodies (IgM and IgG) collected from sheep in 9 governorates **in relation to age, sex, season and housing.**

Localities	Sheep													
	Sex				Age									
	Male		Female		6m-1y		1-2y		2-5y		Up To5y		Total	
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Aswan	-	2	**5**	14		5	-	6	**5**	3	-	2	**5**	16
Draw	-	-	-	3	-	-	-	-	-	3	-	-		3
Edfo	-	5	**3**	25	-	8	-	12	**3**	7	-	3	**3**	30
Nase El-Noba	-	2	-	13	-	4	-	7	-	4	-	-		15
Komobo	-	5	-	20	-	4	-	10	-	9	-	2		25
Elsbayea	-	1	-	5	-	-	-	3	-	3	-	-		6
Abu-Smbl	-	-	-	-	-	-	-	-	-	-	-	-		-
Red Sea	-	3	-	27	-	2	-	8	-	20	-	-		30
Alwadi El-	-	**1**	-	24	-	3	-	14	-	7	-	1		25

Gdid														
Marsa Matroh	-	5	-	25	-	5	-	10	-	12	-	3		30
North Sini	-	2	-	36	-	4	-	14	-	16	-	2		38
South Sini	-	1	-	9	-	1	-	5	-	3	-	1		10
Seuze	-	-	-	5	-	-	-	2	-	3	-	-		5
Ismailia	-	1	-	9	-	-	-	4	-	6	-	-		10
Port Said	-	-	-	5	-	-	-	1	-	4	-	-		5
Total(256)		**28**	**8**	**220**	**-**	**36**	**-**	**96**	**8**	**100**	**-**	**14**	**8**	**248**

Eight out of 256 sheep samples were positive for RVF antibodies by ID screen® RVF competition multispecies ELISA kits. These samples were obtained from 8 (3.13%) females of different ages under category of 2-5years at Aswan and Edfo district with no case history of previous vaccination for all animals under study.

Table. 6. Results of ELISA test for detection of RVFv antibodies (IgM and IgG) collected from goats in 9 governorates **in relation to age, sex, season and housing.**

Localities	**Goats**													
	sex				**Age**									
	male		**female**		**6m-1y**		**1-2y**		**2-5y**		**Up to5y**		**total**	
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Aswan	-	3	-	14		5	-	9		3	-	-		17
Draw	-	2	-	6	-	3	-	5	-	-	-	-		8
Edfo	-	5	**5**	15	-	3	-	7	**5**	5	-	5	**5**	20
Nase El-Noba	-	3	-	9	-	4	-	4	-	4	-	-		12
Komobo	-	1	-	9	-	5	-	6	-	-	-	-		10
Elsbayea	-	1	-	5	-	-	-	1	-	4	-	-		6
Abu-Smbl	-	-	-	-	-	-	-	-	-	-	-	-		-
Red Sea	-	5	-	15	-	-	-	12	-	8	-	-		20
Alwadi El-Gdid	-	-	-	10	-	2	-	7	-	1	-	-		10
Marsa Matroh	-	1	-	19	-	2	-	7	-	10	-	-		20
North Sini	-	7	-	25	-	5	-	13	-	11	-	3		32
South Sini	-	1	-	4	-	2	-	-	-	3	-	-		5
Seuze	-	-	-	-	-	-	-	-	-	-	-	-		-
Ismailia	-	-	-	5	-	-	-	-	-	5	-	-		5
Port said	-	-	-	3	-	-	-	2	-	1	-	-		3
Total		**29**	**5**	**139**	**-**	**31**	**-**	**73**	**5**	**55**	**-**	**8**	**5**	**168**

Five out of 173 goat samples were positive for RVF antibodies by ID screen® RVF competition multispecies ELISA kits. These samples were obtained from 5(2.9%) females of different ages under category of 2-5years at Edfo district with no case history of previous vaccination for all animals under study. By ELISA Using primers RVFV RT-PCR was carried out to amplify 390 bp RNA fragment to confirm the presence of the virus in the positive serum samples. **All 64 seropositive samples by using IDvet screen® France RVF competition multispecies ELISA kits all samples and vector samples were rt-PCR negative**

4- Discussion

The total serum samples from all species were 930 (259 cattle, 242 camels, 256 sheep and 173 goats) were found negatives for the detection of anti-Rift valley fever nucleoprotein (NP) IgM antibodies. The same serodiagnostic studies were done on serum samples which were collected from non- vaccinated animals (sheep, goat, cattle, buffaloes and camels) from El-Qalyubia, El-Dakahlia, El-Sharqia and Kafr El-Skeikh by using commercial competitive ELISA kits for detection of IgG and IgM antibodies against Rift valley fever virus. In the study, the results were 4.52%, 18.5%, 29.5%, and 14.5% respectively. Moreover, IgM antibodies were not detected in the collected serum samples by using IgM ELISA **(Marwan et al., 2012) .** The field samples were collected from mainland France for the known-negative sera (cattle = 191, goats = 119, sheep = 192) and from ruminants of a French overseas territory (Mayotte) for the known-positive sera. A cut-off value of 43% was determined for all species, achieving a sensitivity and specificity of 100% and a concordance of 100% with the species-specific threshold recommended by the manufacturer. The results demonstrate that ELISA may be a suitable diagnostic tool for disease surveillance programs and import/export veterinary certification of French cattle, goats and sheep. **(Cêtre-Sossah *et al.*,2009).**

The collected serum samples were investigated by using RVF competition multi species ELISA evaluated for total anti-RVFV IgM / IgG antibodies using ID screen® RVF competition multispecies ELISA kits. The results showed that all samples were carried out 64/930 (9.3%) RVFV antibodies positive, cattles10/259 (3.75%), camels 41/242 (16.9%), sheep 8/256 (3, 13%) and goats 5/173 (2.9%). Serological surveillance of RVFV among sacrificial animals and their human contacts were carried out using ID screen® RVF competition multispecies

ELISA kits to detect total anti-RVFV IgM/IgG, seropositive samples were tested for specific anti-RVFV IgM antibodies using ID screen® RVF IgM ELISA kits All samples that tested positive for total RVFV antibodies but negative for IgM were assumed to be positive for anti-RVF IgG antibodies An RVF competition multi-species ELISA detecting anti-RVFV IgG/ IgM antibodies and an RVF IgM-specific ELISA were used for serological investigations, 84 (16.8%) of the 500 sacrificial sheep and goats tested seropositive in the competition ELISA but no IgM antibodies were detected in the IgM-specific assay (**Mohamed et al., 2014).** study was carried out on 1186 camels of different ages, sex and in different seasons from 2002-2005 at Assuit villages and Daraw (Aswan). The study revealed that the percentage of RVF anti bodies in sera from camels at Assuit villages were 18.2%, 15.2%, and 20.29% using SNT, CFT and ELISA tests, respectively. While in Aswan (Daraw quarantine) the percentage RVF antibodies as 14.75%, 11.72% and 16.86%, respectively using SNT, CFT, ELISA tests, respectively, **(Faheem, 2006).** The prevalence of RVF antibodies among cattle by IDvet screen® France RVF competitive multispecies (IgG) ELISA kits in relation to age, sex, season and housing. The results showed samples were carried out 10/259 (3.75%) are positive only in females while male were negative. The positive female samples high in age ranged 2-5 years 5/113 with percent 4.3%.The prevalence in cattle 22% **(Elian, 1983).** Examination a total of 1079 serum samples randomly collected from sheep, goats, cattle and buffalo of known and unknown vaccinated status, from different localities in Ismailia province, Sera were subjected to ELISA and serum neutralization test (SNT) for detection of IgG. The positive rate of IgG was 21.1%, 12.3%, 11% and 5% for non-vaccinated sheep, goats, cattle and buffaloes respectively, with an overall prevalence of 14.5%, Seropositive in male and agree with high prevalence of RVF antibodies increased with aged animals **(Youssef, 2004).** Forty one out of 242 camel samples were positive for RVF antibodies by ID screen® RVF competition multispecies ELISA kits. These samples 41 (16.9%) were obtained from males of different ages at Daraw and Abu-smble. The prevalence of RVF antibodies among camels by IDvet screen® France RVF competition multispecies (IgG) ELISA kits in relation to age, sex, season and housing, The high percent of positive samples

were in age range between 2-5 years old 36/242(14.9%). The study was carried out on 1186 camels of different ages, sex and in different seasons from 2002-2005 at Assuit villages and Daraw (Aswan). The study revealed that the percentage of RVF anti bodies in sera from camels at Assuit villages were 18.2%, 15.2%, and 20.29% using SNT, CFT and ELISA tests, respectively. While in Aswan (Daraw quarantine) the percentage RVF antibodies as 14.75%, 11.72% and 16.86%, respectively using SNT, CFT, ELISA tests, respectively (**Faheem, 2006)**.

Eight out of 256 sheep serum samples and Five out of 173 goat serum samples were positive for RVF antibodies by ID screen® RVF competition multispecies ELISA kits. These samples were obtained from 8/256 (3.13%) females under category of 2-5years from sheep at Aswan , Edfo district and 5(2.9%) females under category of 2-5years from goats at Edfo district with un- vaccination history. An active surveillance of RVF in Senegal and Mauritania was conducted from July to November 2002 where IgG antibodies were detected in sera from 785 small ruminants **(FAO, 2002).** A serosurvey was conducted in 2008 on small and large ruminants, the presence of IgG and absence of IgM against RVFV were detected in 887 cattle (25.8%) and 244 small ruminants (24.7%) samples **(Jeanmaire et al., 2011).**

The serum samples and vector were completely negative and **Under-detection limit 0.006639** (**CT value (20.78)** after 20 cycle which indicates that there is no viral RNA in the examined samples. real time PCR are very sensitive and very specific in detection of RVF viral RNA in serum samples and tissue collected from animals and vector.Results obtained of virus isolation from insect revealed that there is no circulating virus in the collected samples from Aswan governorate during the study. rt-PCR technique have been used for detection of RVFV when the availability of virus isolation is restricted **(Sall et al., 2001; Bird et al., 2007)**.To confirm that the presence or absence of RVFV in the positive serum samples, rt-PCR using primers targeting the S segment was carried out.

Conclusion

Rift valley fever is an endemic viral disease in Egypt has zoonotic important which affected human and livestock. The main gate of introduction of infection and outbreaks science 1977 till now from the southern border of Egypt (Aswan governorate). Laboratory diagnosis as ELISA

test for RVF antibodies detection for IgG and IgM and rt-PCR for RNA detection is one of sensitive and specific laboratory tools. The control of rift valley fever must be under restricted steps include obligatory vaccination by local inactivated vaccine, vector control, annually serosurvellance for evaluation of the vaccination process and public health education

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