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Rift Valley Fever Virus (RVFv) Dissemination inside Mosquitoes and Investigation of the Influence of Climate on Mosquitoes Abundance

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ABSTRACT

One year surveys were conducted at two sites in Khartoum State capital of Sudan: Hellat Kuku and Soba West. The study was focused into two species of mosquitoes: *Aedes vexans* and *Culex quinquefasciatus.* This selection was based on previous investigations indicated their being positive for RVF. The study aimed to investigate on RVFv dissemination inside two species mosquitoes in Khartoum State (Sudan) and investigation of the influence of climate on mosquitoes abundance. Weekly aspiration of wild mosquitoes has been conducted during 1st July 2011- 1st July 2012. The collected mosquitoes were identified by using classical keys. Data of climate were obtained from Sudan Meteorology Authorities. Males and females of the two mosquitoes species were divided into three cohorts: salivary glands, abdomen, and wings and legs. RVF Virus was therefore examined into twelve pools using Real- Time PCR technique. Results showed that Temperature, precipitation, and RH were significantly associated with the number of aspirated mosquitoes. The virus has been detected inside the pools of the abdomen and salivary glands of *Ae. vexans* mosquitoes. However, pools of salivary glands of *Cx. quinquefasciatus* were negative of the virus in spite of dissemination of the virus in the wings and legs. Based on these results it can be concluded that climatic factors affected on the number of aspirated mosquitoes during the study period. *Ae. vexans* mosquitoes exhibited an indicator of being competent to transmit the virus in contrast to *Cx. quinquefasciatus*.

Keywords: Arboviruses, Vectors, Competence, Sudan
Abbreviations: Rift valley Fever virus (RVFv), *Culex quinquefasciatus* (*Cx. quinquefasciatus*), *Aedes vexans* (*Ae. vexans*), National Ministry of Health (NMoH), Relative humidity (RH).

1. Introduction

This study was aimed to investigate on Rift Valley Fever Virus (RVFv) dissemination inside two species of mosquitoes in Khartoum State (Sudan) besides investigation on the influence of climate on mosquitoes abundance. Rift Valley Fever (RVF) is caused by a zoonotic arbovirus which belongs to the genus *Phlebovirus* within the family *Bunyaviride*. It can be transmitted to humans and livestock by many routes such as aerosols and direct contact with blood and fluids of infected animals [1]. Although the RVF virus natural infectious cycle has been linked to the *Aedes sp.,* high levels of viremia in animals which would lead to infection of secondary vector species and finally other mammals and livestock infection of animal by the virus is reported to cause abortions and death in susceptible animals [2]. In humans, RVF causes a severe influenza-like disease, occasionally accompanied with more serious effects such as haemorrhagic complications, hepatitis, encephalitis, blindness and sometimes death [3]. Global burden of RVF is unclear because RVF outbreaks are neither seasonal nor annual, but linked with periods of unusual high rainfall and the virus can be transferred transovarially from females to eggs in some mosquito species of the genus *Aedes* [4]. The first RVF outbreak in Sudan was recorded in 1973 in the Kusti area; the second occurred in 2003. During the second outbreak, 90 endogenous cases of RVF were recorded in Khartoum State [5].The most recent RVF outbreak in Sudan occurred in 2007 and 2008, the recorded number of RVF human cases in Sudan, have reached; 566, 698 with 222 and 178 deaths respectively [5,6]. During this outbreak 150 endogenous cases of RVF were recorded in Khartoum State [5].

Neither effective treatments nor humans' vaccines are presently available for the virus, thus vector control is considered to be the most effective method for the prevention and control of the disease [7]. Arboviruses infect the mosquito midgut following ingestion of a viremic blood, replicate, disseminate to the salivary glands, and emerge into saliva to be transmitted when the mosquito bites [8]. The presence of the virus in salivary glands and its dissemination from the bodies of mosquitoes are crucial to understand the transmission possibility of the virus by the competent mosquito vectors. According to this information the study of the vector competence is undertaken. On the other hand assessments of other factors which influence the transmission of RVF such as measurement of the efficacy of implemented control measures against non-malaria vectors in Khartoum State are of significant importance to design an efficient vector control to limit transmission as soon as the first animal and/or human cases are diagnosed. Therefore, surveillance of the fluctuations in the abundance of adult mosquitoes, number of breeding and resting sites (in relation to their proximity to houses and animal shelters), and incidence of RVF constituted the core of this research. Mosquito control maps may include control areas and non- sprayed areas within the flight range of mosquitoes from different breeding sites and habitat locations. Adult and larvae mosquito surveillance furnishes data to determine the best times and places for adulticide and larvicide spraying [9]. This data is also used to check results of treatments and for reporting to the public and officials the extent of the problem. Interpreting adult and larvae mosquito surveillance records and reports helps translate this information into action regarding manpower, material, equipment, insecticides, and furnishes justification for the entire operation [10].

The main aim of this research was to investigate on to investigate on RVFv dissemination inside two species of mosquitoes in Khartoum State (Sudan) besides investigation on the influence of climate on mosquitoes abundance.

2. Materials and Methods:

Khartoum state is the most amplifying city in Sudan. The area lies within the poor savannah region, characterized by a short rainy season (July to September), a short winter (November to February) and a relatively longer summer. People from different tribes and geographical areas have settled in Khartoum, some of them displaced from hyper endemic/mesoendemic zones. The civil wars at some regions of Sudan as well as the fluctuating socio-economic status of the population contributed adversely to the increment of these migrations. This unplanned and uncontrolled urbanization produces ideal conditions for increased transmission of mosquito-borne diseases. A Prospective study conducted at Soba West and Hellat Kuku during 2011-2012. Soba West is 15 km South from Khartoum 15, 5036 in latitude (1530'12.960"N), and 32,6461 (3238'45.960"E in longitude). Livestock prevalence in Soba West during the study was 33% Cows, 50% Goat, and 17% Sheep. Those animals were recorded as susceptible hosts of RVF virus [11]. Positive RVF *Aedes vexans* mosquitoes were collected from this study site [11]. The existence of the susceptible animal hosts as well as the occurrence of the flood water mosquito *Aedes vexans* raised the risk of circulation of the virus between animals and humans through these mosquitoes. Hellat Kuku lies in Khartoum North, North eastern to the Nile. It is characterized by farms and irrigation schemes. The soil is clay and the topography of the ground is characterized by numerous pot holes. These constitute potential breeding sites of mosquitoes. Livestock prevalence in this site during the study period was 53% Goat, 5% Camels, and 42% Cows. Nomads usually settle in the area, they travel from eastern Sudan to Khartoum in August, then return to the east in January. Wild specimens of *Culex quinquefasciatus* collected from this site were positive for RVF virus during previous investigation [11]. Ethical clearance and national endorsement for this research were obtained from the ethical committee of NMoH- Sudan. Residents in the two study sites were asked to sign informed consent forms before the beginning of the study. Stations were determined according to the presence of risk factors of RVF in specific locations at the two study sites, namely: presence of breeding and resting sites of mosquitoes, proximity of those sites to human dwellings and animal shelters. Populations of males and blood-fed *Aedes vexans* and *Cx. quinquefasciatus* were collected from Khartoum Zone (Soba West and Hellat Kuku respectively). Certain method [10] was adopted for the construction of mosquitoes collection maps with slight modifications. The probable mosquito-resting places in a specific area by means of a rapid reconnaissance survey were spotted and their geographic position was recorded with a GPS to compute their positions in relation to the houses and animal shelters in the area. These places were carefully designated and marked on a map, then the specific species breeding and resting habitats were determined and permanent sampling stations were established. All larval and adult breeding and resting sites were identified by symbols or numbers. Adult mosquitoes counts made at these stations at weekly intervals (once a week). Number of mosquitoes collectors as well as the collection period were constant each time.

The adult mosquitoes which aspirated from outside resting sites (stations for collection of adult mosquitoes) then separated according to their genera into labeled cups covered with a piece of mosquito net. A small slit was made at the middle of each net and covered with a piece of moist cotton to prevent mosquitoes escaping during aspiration. Adult mosquitoes were identified to species level using morphological keys [12], and preserved in sterile cryovials at -40 ^{0}C freezer for the virus screening. Well preserved specimens of the collected adult males, blood-fed and gravid female mosquitoes representing the two targeted species were selected for RVF virus surveillance. A total of 2487 specimens (divided into twelve pools) have been examined for RVFV using Real- time PCR technique. The number of specimens per pool was recommended in the literature [13] which mentioned that RT-PCR is sensitive enough to detect reliably a single RVFV-infected mosquito in pool sizes as large as 600 mosquitoes and probably as large as 16,000 mosquitoes. Monthly and weekly data (from July 2011- July 2012) of precipitation (mm) and (RH% and Temperature) respectively were collected from Sudan meteorology authorities. Number of the collected specimens of the two species: *Cx. quinquefasciatus* and *Ae. vexans* was plotted against each of the above climatic factors and the results were then analyzed using SPSS 2006 and NCSS 2007 software. After the morphological identification of the field-collected males and blood-fed female mosquitoes each specimen was individually divided into three parts then the specimens were separated into pools represented: Abdomens, wings and legs, salivary glands of *Cx. quinquefasciatus* specimens (for both males and blood fed females of mosquitoes). The same pools were prepared from the specimens of *Ae. vexans* (dissection of mosquitoes specimens was performed over cool and dry ice bags in order to prevent virus lose). As a result of this twelve pools were then assayed for the RVF virus using Real Time PCR technique. The number of mosquito specimens in each pool

was determined according to the availability of mosquitoes during the surveys. Each pool was triturated separately with a mortar and a pestle in varying volumes of Virus Transport Medium (VTM) depending on the group size as follows: 10 - 50 mosquitoes (2.0 ml), 100 mosquitoes (3.0 ml), 150 mosquitoes (4.0 ml), 200 mosquitoes (5.0 ml), and 300 mosquitoes (8.0 ml). The medium was the Leibovitz medium with 10% fetal calf serum, antibiotics, and fungicide. The supernatants were filtered centrifuged at 3,000 cpm for 30 mm then filtered using 0.45µm syringe filters. The supernatants were used for the RNA extraction. Viral RNA was extracted from the pools of mosquitoes using the method of [13]. RNA was extracted from clarified supernatant using a BSC52S1 Simply P Total RNA extraction kit (BioFlux, Shanghai, China) according to the instructions of manufacturers. Samples were homogenized and 600 µl of the R2 solution were added then the mixture was incubated at the room temperature for 3-5 minutes. The supernatant was transferred into the spin column, centrifuged for 30 seconds. This step was repeated then centrifuged for an additional 1 minute at 10.000 rpm then the spin column was transferred to a sterile 1.5 ml micro centrifuge tube. 20-50 µl of the Elution Buffer were then added; incubated at room temperature for 30 seconds. The total RNA was stored at -40°C until the RT-PCR was performed. The RT-PCR was performed using the RT-PCR tube. Depending on the number of samples, the Master Mix was prepared as follows: (19 µl of the super mix) X (No. samples). Total numbers of samples (pools) was 12+ 1 positive control+ 1 Negative control+ 1 to balance the possible loss. Therefore 285µl (19x15) were prepared. 1 µl of the enzyme was added x No. samples (1 X15= 15 µl). The total volume of the prepared master mix was: 285 +15 = 300 µl. 20 µl of the master mix was added for each sample (15 samples). The Primer used was an Oligonucleotide specific primer with the Following sequence:

NSca 5'-CCTTAACCTCTAATCAAC-3' Map position: 841 - 824, orientation: Anti- sense.
NSng 5'-TATCATGGATTACTTTCC-3' Map position: 31 - 48, orientation: Sense.

The PCR tubes were closed and transferred into the rotor of the Rotor Gene TM instrument. Denaturation occurred at 95°C (for 5 s), one step annealing and extension occurred at 57°C (for 35 s). The following steps were the cycling for amplification of cDNA (the total number of cycles was 45). Then, the sensitivity of the fluorescence channels was adjusted. Finally, the Rotor Gene TM run was initiated.

3. Results:

3.1 Mosquitoes Abundance in the Collection Stations:

Seventeen foci were representing resting and breeding sites of *Cx. quinquefasciatus* mosquitoes in Hellat Kuku while four foci were representing breeding and resting sites of *Ae. vexans* mosquitoes. Location of each of the foci has been determined using GPS. Number of adult *Cx. quinquefasciatus* mosquitoes collected from the stations at Hellat Kuku over the study period was 3475 (Mean: 72 adult mosquitoes/ week) while number of adult *Ae. vexans* mosquitoes collected from the stations at Soba West during the study period was 425 (Mean: 9 adult mosquitoes/ week). Ratio of *Ae. vexans: Cx. quinquefasciatus* abundance was 1: 8 (Table 1).

Table 1: Weekly Density of Mosquitoes Collected from the Stations at the Two Study Sites (1st July 2011- 1st July 2012)

Months	Weeks	*Cx. quinquefasciatus*		*Ae. vexans*	
		Adults	Adults%	Adults	Adults%
July	1	115	3.31	0	0.00
	2	39	1.12	2	0.47
	3	41	1.18	85	20.00
	4	1	0.03	2	0.47
August	1	196	5.64	89	20.94
	2	50	1.44	23	5.41
	3	232	6.68	9	2.12
	4	129	3.71	1	0.24
September	1	103	2.96	0	0.00
	2	124	3.57	5	1.18
	3	58	1.67	0	0.00
	4	77	2.22	0	0.00
October	1	73	2.10	0	0.00
	2	123	3.54	0	0.00
	3	140	4.03	9	2.12
	4	5	0.14	4	0.94
November	1	103	2.96	12	2.82
	2	73	2.10	12	2.82
	3	68	1.96	8	1.88
	4	42	1.21	9	2.12
December	1	27	0.78	8	1.88
	2	75	2.16	2	0.47
	3	54	1.55	20	4.71
	4	70	2.01	7	1.65
January	1	17	0.49	9	2.12
	2	113	3.25	5	1.18

	3	55	1.58	2	0.47
	4	16	0.46	3	0.71
February	1	17	0.49	13	3.06
	2	113	3.25	15	3.53
	3	55	1.58	2	0.47
	4	16	0.46	0	0
March	1	28	0.81	0	0
	2	37	1.06	0	0
	3	82	2.36	2	0.47
	4	113	3.25	0	0
April	1	64	1.84	3	0.71
	2	37	1.06	0	0
	3	26	0.75	12	2.82
	4	113	3.25	3	0.71
May	1	57	1.64	7	1.65
	2	37	1.06	4	0.94
	3	64	1.84	3	0.71
	4	52	1.50	22	5.18
June	1	103	2.96	3	0.71
	2	52	1.50	6	1.41
	3	80	2.30	4	0.94
	4	110	3.17	0	0

The peak collected *Cx. quinquefasciatus* and *Ae. vexans* mosquitoes was during week five of the study (First week of August): 5.6% and 20.9% respectively.

3.2 Mosquitoes abundance and climatic factors:

Weekly average temperature and Relative humidity during the study period were investigated. The average temperature, relative humidity was 30.7^0 C, 24.4% (Table 2).

Table 2: Weekly climatic mean values for Khartoum State (1st July 2011- 1st July 2012)

Months	**Weeks**	**T** (ºC)	**RH** (%)
July	1	35.1	18.6
	2	35.4	24.6
	3	35.4	28
	4	31.5	47.4
August	1	30.8	53.6
	2	30.6	54.3
	3	32.5	44.7
	4	33.13	42.1
September	1	32.4	43.9
	2	31.93	47.9
	3	33.5	33
	4	33.84	28.4
October	1	34.5	31.1
	2	34	30.6
	3	33.7	33.1
	4	31.98	25
November	1	28.2	18.7
	2	28.4	21.7
	3	27.1	25.4
	4	23.1	25.8
December	1	24.1	27.4
	2	25.2	32.1
	3	24.8	27.4
	4	26.7	29.6

January	1	22.9	23.3
	2	20.4	20.4
	3	21.9	19.4
	4	24.3	24
February	1	27.1	23.3
	2	29.2	26.6
	3	28.9	14.1
	4	27.25	15.4
March	1	24.7	11.4
	2	33.3	11.1
	3	27.9	11.9
	4	28.8	7.8
April	1	31.5	9.9
	2	33.9	7.7
	3	35.5	7.6
	4	33.3	6
May	1	34.9	7.9
	2	36.2	8
	3	36.99	8.3
	4	36.1	24.1
June	1	36.97	19.7
	2	36.4	16.6
	3	36.6	16.6
	4	32.6	37.5

T: Mean Temperature (°C) **RH:** Mean humidity (%)

Monthly precipitation data were collected for the twelve months of the study. A total of 120 mm were recorded over the twelve months of the study. The peak precipitation has been recorded during August (53.1 mm) (Table 3)

Table 3: Monthly average rain fall parameters in Khartoum state (1st July 2011- 1st July 2012)

Months	**Average rain fall parameters**	
	Precipitation (mm)	**Average rainfall days**
July	24.3	5
August	53.1	7
September	25.4	1
October	6.4	1
November	0.3	1
December	0.0	0
January	0.0	0
February	0.0	0
March	0.1	1
April	0.4	1
May	5.3	1
June	4.7	1
Total	120	19

Statistical analysis showed significant correlation between RH and abundance of adult *Cx. quinquefasciatus* mosquitoes (r= 0.376, P. value= 0.008 (Spearman correlation). *Ae. vexans* mosquitoes also showed significant correlation with (RH) (r= 0.323, P. value= 0.025) (Pearson correlation). Statistical analysis using T-Test showed that there was high abundance of *Cx. quinquefasciatus* mosquitoes in temperature above 25°C than in temperature below 25°C (Significant different by T- Test independent sample):

Mean *Cx. quinquefasciatus* mosquitoes when T> 25 °C= 78 mosquitoes/ week (Temperature Range 25°- 36.99°C).

While Mean *Cx. quinquefasciatus* mosquitoes when T≤ 25°C= 44 mosquitoes/ week (Temperature Range 20.4 °- 25°C).

Statistical analysis also indicated that there was lower abundance of *Ae. vexans* mosquitoes in temperature> 31°C than in temperature≤ 31°C (Man- Whitney test):

Median *Ae. vexans* mosquitoes (T> 31 °C)= 3 mosquitoes/week (Temperature Range 31°- 36°C).

Median *Ae. vexans* mosquitoes (T≤ 31 °C)= 8 mosquitoes/week (Temperature range 20.4°- 31°C) .

Statistical analysis also showed that there was higher abundance of *Cx. quinquefasciatus* mosquitoes when the RH was more than 32% (Significant different by T- Test independent sample):

Mean *Cx. quinquefasciatus* mosquitoes when RH> 32%= 55 mosquitoes/ week (RH Range 32% - 54%).

Mean *Cx. quinquefasciatus* mosquitoes when RH≤ 32%= 11 mosquitoes/ week (RH Range 6% - 32%).

Furthermore statistical analysis showed that there was higher abundance of *Ae. vexans* mosquitoes when RH increased over 20% (significant difference by Man- Whitney test):

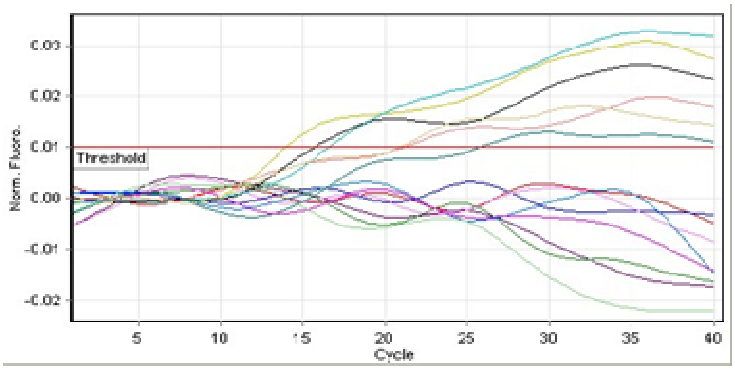
Median *Ae. vexans* mosquitoes when RH> 20%= 7 mosquitoes/week (RH Range 21% - 54%).

Median *Ae. vexans* mosquitoes when RH≤ 20%= 3 mosquitoes/week (RH Range 6% - 20%).

There was significant correlation between precipitation and the abundance of *Cx. quinquefasciatus* and *Ae. vexans* mosquitoes (r= 0.6and 0.8 respectively). According to the information of NMoH Sudan no adult control was implemented during the study period. Larviciding was applied homogenously four times a month (once a week) at the two study sites thus the possibility that larviciding could be a variant influencing the fluctuation in the abundance of mosquitoes was ruled out.

3.3 Virus Dissemination inside Pools of Mosquitoes:

A total of 829 adult mosquitoes were examined for RVFV using Real- Time PCR technique, divided into the following twelve pools as follows (Fig 1):



No.	Colour	Name	Type	Ct
1		Negative Control	NTC	
2		Positive control	PTC	14.02
3		1	*Ae. vexans*♂ wings and legs (86 specimen)	
4		2	*Ae. vexans*♂ Abdomen (86 specimen)	
5		3	*Ae. vexans*♂ Salivary glands (86 specimen)	
6		4	*Ae. vexans* ♀ wings and legs(143 specimen)	
7		5	*Ae. vexans*♀ Abdomen (143 specimen)	25.96
8		6	*Ae. vexans*♀ Salivary glands (143 specimen)	21.30
9		7	*Cx. quinquefasciatus* ♂ wings and legs (300 specimen)	
10		8	*Cx. quinquefasciatus* ♂ Salivary glands (300 specimen)	
11		9	*Cx. quinquefasciatus* ♂ Abdomen (300 specimen)	15.75
12		10	*Cx. quinquefasciatus*♀ wings and legs (300 specimen)	16.68
13		11	*Cx. quinquefasciatus* ♀ Abdomen (300 specimen)	21.10
14		12	*Cx. quinquefasciatus*♀ Salivary glands (300 specimen)	

Fig 1: Detection of RVF virus inside pools of mosquitoes: *Aedes vexans* and *Culex quinquefasciatus* (respectively) using Real-Time PCR

Pool 1: *Ae. vexans* (♂s wings and legs: 86 specimens)
Pool 2: *Ae. vexans* (♂s abdomen: 86 specimens)
Pool 3: *Ae. vexans* (♂s salivary glands: 86 specimens)
Pool 4: *Ae. vexans* (♀s wings and legs: 143 specimens)
Pool 5: *Ae. vexans* (♀s abdomen: 143 specimens)
Pool 6: *Ae. vexans* (♀s salivary glands: 143 specimens)
Pool 7: *Cx. quinquefasciatus* (♂s wings+ legs: 300 specimens)
Pool 8: *Cx. quinquefasciatus* (♂s salivary glands: 300 specimens)
Pool 9: *Cx. quinquefasciatus* (♂s abdomen: 300 specimens)
Pool 10: *Cx. quinquefasciatus* (♀s wings and legs: 300 specimens)
Pool 11: *Cx. quinquefasciatus* (♀s abdomen: 300 specimens)
Pool 12: *Cx. quinquefasciatus* (♀s salivary glands: 300 specimens).
Five pools out of the above twelve pools were positive for RVFV (CT value of the positive control was 14.02):
Pool 5 (CT value 25.96), Pool 6 (CT value 21.3), Pool 9 (CT value 15.75), Pool 10 (CT value 16.68), Pool 11 (CT value 21.1).

4. Discussion:

Aedes vexans arabiensis (Megan) and *Culex quinquefasciatus* (Say) mosquitoes are potential vectors of RVF. *Aedes vexans* is one of the flood water- breeding species which is considered as one of the potential vectors of RVF in West Africa [14]. *Culex quinquefasciatus* was also recorded as a vector of RVF in Kenya [15]. The latter species is also suspected to have a role in human to human transmission [16]. Due to the ongoing climate changes and reappearance of some zoonosis that were previously considered eradicated, there is a growing concern about potential disease outbreaks. Therefore, the prediction of increased adult population abundances becomes an essential tool for the appropriate implementation of mosquito control strategies. In order to describe the population dynamics of *Ae. vexans* and *Cx. quinquefasciatus* mosquitoes in temperate climate regions, a one-year period (2011-2012) climate-dependent surveillance was conducted. In order to investigate the dissemination of RVFV inside the two mosquito species and to shed light on factors that possibly influence the

abundance of the two species, specific entomological surveys have been conducted at two study sites of Khartoum State (Hellat Kuku and Soba West) between 1stJuly 2011–1stJuly 2012. *Culex. quinquefasciatus* mosquitoes were collected from Hellat Kuku while *Aedes vexans* were collected from Soba West. These study sites as well as the two species of mosquitoes were purposely selected for this study due to the fact that RVFv has already been detected inside pools of populations of blood-fed *Ae. vexans* and *Cx. quinquefasciatus* (collected from Soba West and Hellat Kuku respectively) [11]. Susceptible animal hosts such as sheep, goats, and cows were also found in the two study sites. High abundance of mosquitoes collected from the two study sites during recent studies [11, 17] indicated deficiency and gaps in the implemented vector control measures in the area. Furthermore detection of the virus in such an arthropod vector is not crucial for incriminating this species as a competent vector capable for transmitting the virus to the vertebrate hosts. Furthermore, the vector control directorate authorities/ National Ministry of Health (Sudan) assumed that the implemented vector control measures at the two study sites are affective to encounter the risk of the two species of mosquitoes at the two study sites [18]. These points gave the rationale of investigating the factors influenced abundance of the two mentioned species of mosquitoes as well as investigating the dissemination of the virus inside the two species of mosquitoes. Results of this research may therefore assist in signing the actual situation of the risk factors of possible RVF outbreaks in Khartoum State during the coming time and provide evidence base for interventions. This study covered the three seasons of the year (the rainy season between mid- July to the end of October), the post rainy season (beginning of November to the end of March) and the pre rainy season (beginning of April to mid-July). Besides, referring to the records of Epidemiology Directorate/ National Ministry of Health the period of the study was considered as inter-epizootic [18]. Previous investigations focused either on the periods of outbreaks [19] or the periods pre and post to the rainy season only [11, 17]. Stations were constructed at Soba West and Hellat Kuku to cover the potential resting and breeding sites of mosquitoes inside, between, and proximal (within the flight range of mosquitoes) to the houses and animal shelters in the area. Seventeen stations were selected at Hellat Kuku, 3 of which were representing both breeding and resting sites of mosquitoes while 14 were representing resting sites only. At Soba West the *Ae. vexns* mosquitoes were found only in the Veterinary research centre. Four stations were constructed there, 1 of them was a breeding site, 2 were resting sites, and one of them was a site for both breeding and resting of mosquitoes. Both permanent and temporal resting sites of adult mosquitoes were involved in this study and considered as fixed stations for the entomological surveillance during the whole year of the investigation. The location of these stations as well as their altitude and longitude were determined and remarked on electronic maps using GPS and Google Earth satellite images. All stations at Hellat Kuku were localized between houses and animal shelters while at Soba West the farthest station was 200 m away from houses and animal shelters. All the collection stations were constructed outdoors for two reasons: (i) To minimize nuisance of the residences inside their houses particularly early in the morning (time of collection of *Cx. quinquefasciatus*) (ii) To monitor the variant vector control interventions outdoors (e.g. larviciding, biological control, source reduction etc.) and their impact on the abundance of mosquitoes. The number of adult mosquitoes in these stations were monitored weekly (once time a week) for a period of twelve months starting from the beginning of July 2011, persisted till the 1st July 2012. Further investigations have been done to determine the factors influenced the fluctuation in the abundance of mosquitoes. The factors hypothesized to have that impact, namely were the climatic factors. Statistical analysis showed significant correlation between precipitation and the abundance of *Cx. quinquefasciatus* and *Ae. vexans* mosquitoes. The maximum flight range of adult *Ae. vexans* mosquitoes is 25 to 30 miles [20]. The stations for the collection of these species of mosquitoes were two miles far from the borders of Al Jazeera State. This means that outbreaks of RVF in Al Jazeera State can be disseminated by the *Ae. vexans* mosquitoes at Soba West. Furthermore, during the last outbreak of RVF in Sudan; the highest proportion of the recorded cases were recorded from Al Jazeera State (61.3%) followed by White Nile (16.2%) then Sinnar (10.0%) and Khartoum State (5.9%) [5]. This indicates that the risk factors of RVF are found at Al Jazeera State more frequently than other States. This increases the risk of disseminating the outbreak of RVFV from Al Jazeera State to Soba West. Statistical analysis using T-Test showed that there was high abundance of *Cx. quinquefasciatus* adult mosquitoes in temperature above 25 ºC than in temperature below 25ºC (Significant different by T- Test independent sample): Mean *Cx. quinquefasciatus* adult mosquitoes when T > 25 ºC= 78 mosquitoes/ week. Contrary to this; Statistical analysis showed that there was lower abundance of *Ae. vexans* adult mosquitoes in temperature > 31ºC than in temperature ≤ 31 ºC. That means *Ae. vexans* mosquitoes cannot tolerate high temperature. This result coincides with the findings of a research [21] reported that development time decreased with each increase in rearing temperature in *Aedes vexans* until the optimum survival temperature was reached. The optimum survival temperature for *A. vexans* was 26.5 °C. Another study [22] revealed that survival of *Cx. quinquefasciatus* mosquitoes from immature stages to adult emergence was highest in the range from 20º to 30 °C. Statistical analysis also showed that there was higher abundance of *Cx. quinquefasciatus* adult mosquitoes when the RH was more than 32%. Furthermore statistical analysis showed that there was higher abundance of *Ae. vexans* adult mosquitoes when RH increased over 20%. These results coincide with the findings of a research mentioned that rainfall significantly influenced the population of *Cx. quinquefasciatus* ($t=2.63$; $P<0.05$). Fed group and semi-gravid group showed a strong correlation with the relative humidity ($P<0.05$), rain fall ($P<0.05$) [23]. Intra thoracic injection of the virus inside mosquitoes was difficult to be applied *in vitro* in Sudan due to the absence of laboratories with bio safety level three or above therefore an alternative method that gave clues of the competence of the two species of mosquitoes to transmit the virus was implemented. Dissemination of the virus has been investigated inside three cohorts of mosquitoes: Salivary glands, abdomen, wings and legs. These cohorts represented *Cx. quinquefasciatus* mosquitoes (males and females) collected from Hellat kuku and *Ae. vexans* mosquitoes (males and females) collected from Soba West. This resulted into 12 pools. The number of specimens in each pool varied according to the availability of the species of mosquitoes during the survey. Five pools out of the 12 were positive when examined for the RVFV using Real- Time PCR technique. The virus was not detected inside males of *Ae. vexans* mosquitoes. This may be due to the minimum number of collected specimens during the survey (86 specimens) while the virus has been detected inside pool 5 and 6 (*Ae. vexans* ♀s Abdomen and Salivary glands respectively) and pool 9 (Abdomen of ♂s *Cx.*

quinquefasciatus). The virus has also been detected inside pool 10 and 11 (*Cx. quinquefasciatus* ♀s Abdomen and (wings and legs) respectively) while the test showed negative result for pool 12 which represented the salivary glands of *Cx. quinquefasciatus* ♀s. These results coincide with results of another research showed that when *Ae. vexans* and *Cx. quinquefasciatus* female mosquitoes exposed to avirulent clone of RVFV the virus was better replicated inside the first species compared to the second one [24]. Another study conducted by the United States Department of Agriculture Supported our findings that RVFV can survive the salivary glands barriers of *Ae. vexans* mosquitoes. The report mentioned that when the dissemination of RVFV has been investigated inside three mosquito species (*Anopheles quadrimaculatus*, *Aedes vexans*, and *Aedes dorsalis*) and one biting midge species (*Culicoides sonorensis*) a lower percentage of *Ae. vexans* became infected and developed a disseminated infection, and however, had a higher rate of virus transmission by bite [25]. Many studies revealed that *Cx. quinquefasciatus* mosquitoes are unable to escape the salivary glands barriers which is in agreement with the findings of this research where the virus has been detected inside pools of the abdomen and body of this species while the virus was absent in the pools of the salivary glands. Our study confirms that RVFV disseminates to the legs of wild *Cx. quinquefasciatus* mosquitoes and suggests that these mosquitoes could play a role in the maintenance or transmission of RVFV in disease-endemic regions. Positive results from testing of the mosquito legs (and salivary glands) also diminish concern about false-positive results from testing whole mosquitoes, which might contain recent blood meals with substantial viral content. The first example is a study confirmed that single mosquito leg samples are sufficient for PCR/qRT-PCR detection of RVFV [26, 27]. Other vector competence studies have shown that RVFV does disseminate in *Cx. quinquefasciatus* mosquitoes but have yet to show that they are efficient vectors for RVFV [15]. Although identification of viral RNA in the legs of *Cx. quinquefasciatus* supports dissemination of virus, no conclusions can be made from these results regarding the role of these mosquitoes in maintaining these arboviruses in this environment or their ability to transmit virus. Additional studies are required to confirm vector competence of *Cx. quinquefasciatus* and *Ae. vexans* mosquitoes tested for the RVFV.

5. Conclusions:

Based on the findings of this study it can be concluded that vector control interventions don't play a role in the fluctuation of the abundance of mosquitoes contrary to climatic factors (Temperature, Relative humidity, and Rainfall). The virus survived the barriers inside *Ae. vexans* mosquitoes and reached the salivary glands in contrast to *Cx. quinquefasciatus*.

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