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Abundance and spatial distribution of *Culex* and *Aedes* mosquitoes as potential vectors of rift valley fever in Jazan region

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Abstract

The mosquito and *Sandfly* fauna of Jazan region-Southwest Saudi Arabia were investigated to identify the density and the geographic distribution of potential vectors of Rift Valley Fever Virus (RVFV), as part of ongoing control program against RVF during inter-epizootic period. Overall, 128190 mosquito females, 3229 *Phlebotomine sandflyies* and 2018 *Culicoides* were collected from 18 October 2015 through 4 Sep 2016, by using a carbon-dioxide (dry ice) - baited light traps. All samples were morphologically identified at genus level and frozen at -80 before submitted to the reverse transcription polymerase chain reaction (RT-PCR) assay to investigate the likely circulation of RVFV in mosquitoes. Among the collected mosquitoes, *Culex* species was consistently the predominant mosquito, ranged from 98.13% to 99.26% in Sabia and Abuareesh respectively. While, *phlebotomus sandflies*, were found sporadically distributed in all districts with high relative abundance in Bulgazi (5.6%). Interestingly, all samples tested by RT-PCR were reported as negative for RVFV. In light of the reported results, conducting more spatio-temporal distribution of mosquitoes based on species identification should be considered in future studies towards implementing more effective control measures against mosquito-borne diseases.

Keywords: Mosquito- Phlebotomus- Culicoides- RVF

Introduction

Rift Valley Fever (RVF) is a serious infectious disease with severe clinical manifestations and health consequences for humans and a wide range of domestic ruminants, RVF is caused by mosquito-borne virus that belongs to the family Bunyaviridae, genus phlebovirus [1]. Virus transmission usually occurs either through the bite by an infected mosquito or by direct contact with infected animal tissues, body fluids or aborted fetal materials [2]. It was first reported among livestock in Kenya in 1931, since then it has been reported as occurring not only in most African countries, but also in the Arabian Peninsula, particularly in Saudi Arabia and Yemen [3, 4]. Outbreaks of RVF have always been associated with periods of heavy rainfall that raises the level of water in dry wooded grasslands and floods the grassland depressions, resulting in increasing of mosquito populations which are thought to play a pivotal role in the life cycle and amplification of the virus during epidemics [5].

Species of mosquitoes that are incriminated as principal vectors and have the potential to transmit RVFV via bites vary from region to region [6]. Several mosquito species serve as principal vectors and able to transmit the virus by bites, most notably those belonging to the genus *Aedes* which is thought to play a significant role in maintaining the endemic city of the disease in the environment through transovarial transmission [7]. In Saudi Arabia, of seven mosquito species detected in Jazan region during the 2000 epidemic, only two species including *Culex tritaeniorhynchus* and *Aedes vexans arabiensis* were confirmed as the principal vectors responsible for the transmission and spreading of RVF virus through animals and humans [8]. The most abundant culicinae mosquitoes collected in Asir region in the same outbreak were *Ae. vexans arabiensis*, *Cx. pipiens complex*, and *Cx. tritaeniorhynchus*. All the previously mentioned mosquito species were considered as an important epidemic and epizootic vectors of RVFV in Saudi Arabia [9].

Since the early recognition of RVF in Saudi Arabia, a comprehensive control program has.

been launched to minimize the spread of the disease in Jazan region. These control measures including but not limited to animal vaccination, vector control, entomological surveillance and virus detection in mosquitoes by molecular techniques. Although these measures contribute significantly to RVF control, the geographical distribution of mosquitoes especially of the genus *Aedes*, based on the geographical coordinates, has not yet been adequately addressed. Moreover, human populations that are more likely to be at risk of biting mosquitoes are not previously identified. Hence, there is a justification for a prospective vector survey covering the whole region to examine the abundance and the distribution of RVF competent vectors, based on geographical locations. This research specifically aim to: (1) study the abundance and the geographical distribution of the potential vectors of RVF in Jazan region and map the locations. (2) to investigate the existence of RVFV in mosquitoes by molecular techniques. (3) to establish mosquito database, that can be used as a platform for controlling emerging mosquito-borne diseases. The results of this study will be useful in identifying high-risk zones, controlling emerging mosquito-borne diseases and enhancing both of control program and surveillance system effectively over the long time.

Materials and Methods

Study Area

Jazan region is located on the farthest south-west Saudi Arabia between longitudes 41°E and 43° E and latitudes 16°N and 18°N, near the Yemeni borders which represent the southern and eastern borders. The Red Sea borders the region from the west for a distance of (330) Km 2 along the sea coast, while Asir region from the north. Jazan region covers an area of 40.457 and KM² and it is divided into 13 governorates and 31centres. The terrain of the region varies and consists of mountain, coastal and fertile plains [10]. The existence of both ecological diversity pattern and different types of vegetation, listed the region among the richest areas in Saudi Arabia with animal biodiversity [11]. The considerable amounts of rainfall, besides the hot humid climate conditions and fields that are irrigated from Wadies, support both of the agricultural and animal production activity which are eventually providing an ideal habitat for RVF vectors.

Mosquito Collection

21 light traps were placed at 375 different locations in six districts with habitats constitute a favorable ecosystem for RVFV, including AL-ardah, Ahed Al-msarha, Sabia, Baish, Balgazi, and Abuareesh). The trap sites were selected based on perceived risk of disease introduction, areas where confirmed or suspected RVF cases were previously reported, outdoor shelters and wild vegetation as well as in the vicinity of Wadies, ponds, dams and sewerage stations. A CO₂ (dry ice)- baited light traps were placed outdoor in the evening approximately one hour before sunset and collected the following morning (from 6:pm to 6:am). Adult mosquito samples were sent to Jazan veterinary laboratory where they were identified morphologically at genus level with a digital microscope by using mosquito keys [12]. All samples were labeled based on genus, sex, GPS location and date. Subsequently, they were stored at -80°C and subjected to virus detection by PCR.

Data Analysis

Relative Abundance (RA %)

The dominance of the mosquito species at each site was estimated by the relative abundance (RA%). This was expressed by the ratio between number of specimens of a species and the total number of specimens of all mosquito species caught in the site $\times 100$ [13].

Pattern of occurrence (C%)

The distribution of mosquito species was estimated using the pattern of occurrence (C%) the ratio between the number of sites positive for the occurrence of mosquitoes and the total number of sites studied [14].

Geospatial Analysis

Different GIS operations and geo-processing tools, including buffer and intersection analysis, were considered to achieve a better understanding of the arboviral incidence. 1.5 Km buffer were processed based on vectors flight range to identify human populations that are likely to be bitten by mosquitoes [15].

Molecular detection of RRVFV

RT-PCR test was conducted for the detection of the virus in mosquitoes as described previously by *Sall et al., (2001)* [16]. A total number of 16300 adult female mosquitoes were pooled in groups of 50 individual mosquitoes at collection site. 326 specimens were processed to obtain a supernatant fluid which was tested by RT-PCR to identify the presence of RRVF viral RNA. Viral RNA was extracted from mosquito homogenates using Trizol-LS reagent according to the manufacturer's instructions. The final RNA pellet was resuspended in 12 μ L of nuclease-free water and then stored on ice or frozen at -80°C. The one-step RT-PCR was performed using the light Cyler RNA Amplification Kit, SYBR Green1, and the light Cyler instrument (ROCHE). The RNA was converted into complementary deoxyribonucleic acid (cDNA). Master mixes were processes based on a ready-to-use LightCycler-RNA Amplification SYBR Green kit (Roche Diagnostics). The cDNA segment of the RRVFV, and the live attenuated RRVF Smithburn strain vaccine Registered NO. G 0124 were used as a positive control.

Results

A targeted vector surveillance in Jazan region was performed from 18 October 2015 to 4 Sep 2016, to examine the distribution and abundance of potential arthropods vectors, at genus level. Out of 128190 adult mosquitoes, 3229 *Phlebotomine sandflyies* and 2018 *Culicoides* were collected in 21 light traps at 375 sites belonging to six districts which include Al-ardah, Abuareesh, Sabia, Almsarha, Bulgazi and Baish. Of 375 sites, *Aedes*, *Culex*, Sandfly, *Culicoides* were collected from (84, 344, 59, 24) sites respectively (table 1). The study indicated that a significant variation in mosquito density was found within districts. Among the collected mosquitoes *Culex* was more abundant and present at all of the sampled sites (91.73%), with RA% ranged from 98.48% in Baish and 91.00% in Bulgazi. In contrast, *Aedes* was recorded in 84 locations, and 29 of these sites (32.8%) had only *Aedes*. In addition, it was found more distributed in Sabia (2.46%), but less common in Baish (0.73%) (table 2). Furthermore, a

total of 3229 *Phlebotomus* and 2018 *Culicoides* were collected during the study period (table3). The highest *phlebotomus* abundance was (5.6%) at Bulgazi, followed by (4.36%) in Almsarha and (3.89%) in Abuareesh. Concerning *Culicoides*, an important Blue Tongue (BT) vector in Jazan region were distributed abundantly in Almsarha (3.13%) and abuareesh (2.18%) but scarce in Sabia (0.02%) and Alardah (.05%), while not reported in Baish (Table 1). The pattern of occurrence was estimated as constant in *Culex* with value of (91.73%) followed by infrequent in *Aedes* (22.13%) and sporadic in both of *phlebotomus* (15.73%) and *Culicoides* (6.4%) (table 2). Most importantly, all samples tested by RT-PCR were reported as negative for RVFV (table 3).

The geospatial analysis estimated that Sandflies were widely distributed in altitudes between 24-760 meter above sea level (Figure 1). Additionally, it was suggested that around (24.486) people in Alardah, Aljadiah, Alaedabi and Bulgazi reside within mosquito flight range and more likely to be

affected by mosquito bites (Figure 2).

Table 1: abundance of mosquito in different districts.

Districts	Relative abundance (RA%)			
	<i>Culex</i>	<i>Aedes</i>	<i>Phlebotomus</i>	<i>Culicoides</i>
Al-ardah	97.27	2.0	0.22	0.51
Abuareesh	93.27	0.73	3.89	2.18
Al-msarhah	91.16	1.35	4.36	3.13
Baish	98.48	0.73	0.79	0
Sabia	95.18	2.46	2.34	0.02
Bulgazi	91.00	1.96	5.6	1.51

Table 2: Pattern of occurrence of mosquito genera in Jazan region.

Mosquito Genera	(C%)	Distribution Pattern(C%)
<i>Culex</i>	91.73	constant
<i>Aedes</i>	22.4	infrequent
<i>Phlebotomus</i>	15.73	sporadic
<i>Culicoides</i>	6.4	sporadic

Table 3: Mosquito Samples tested by RT-PCR.

Districts	Alardah	Abuareesh	Al-msarhah	Baish	Sabia	Bulgazi
Number of Samples	134	84	61	3	11	33
Positive Samples	-	-	-	-	-	-

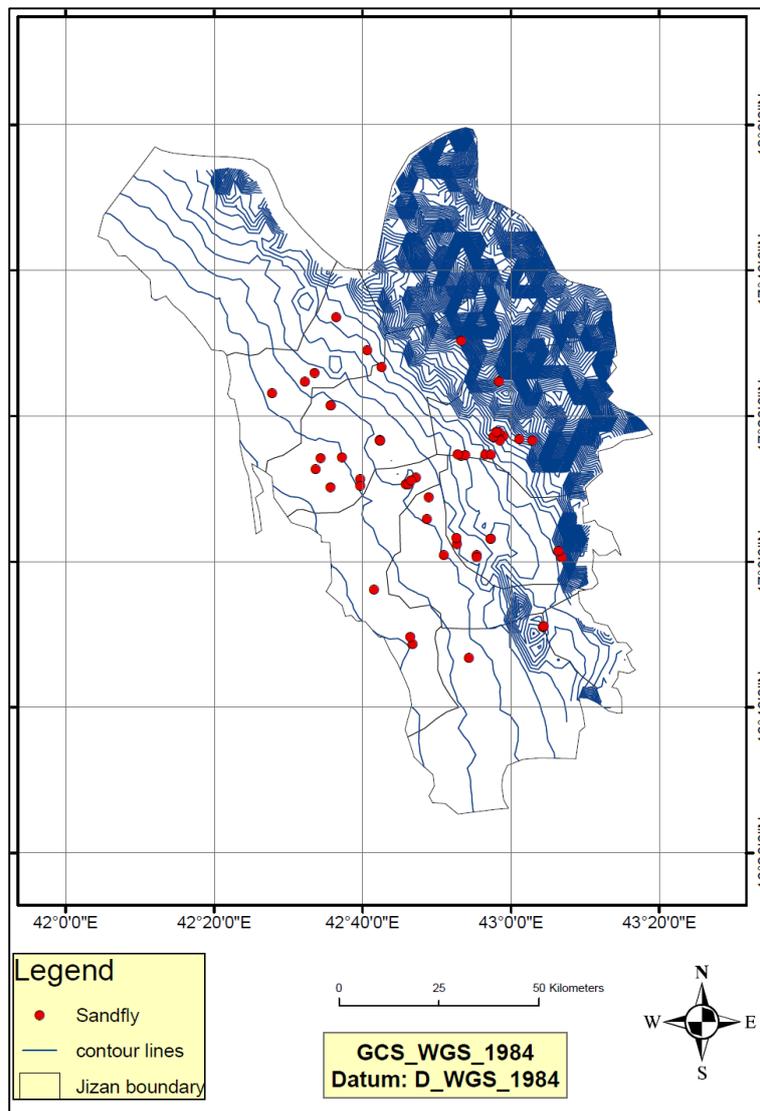


Fig 1: Distribution of Sandflies in Jazan Region

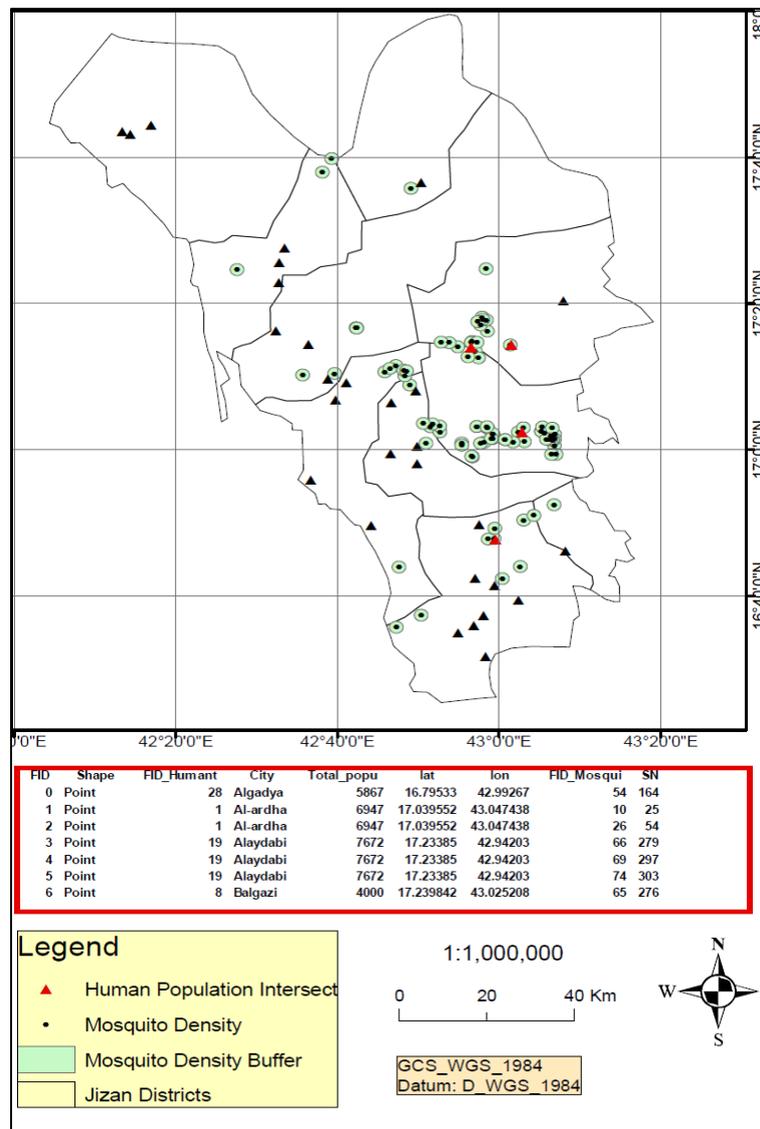


Fig 2: intersection between Human population and Mosquito

Discussion

The present study is an entomological surveillance that was conducted to accurately define the distribution and abundance of RVF competent vectors in Jazan region as an important step in assessing and mapping high risk area that could be prone to RVF outbreaks. The results of the current study revealed that *Culex* and *Aedes* were the most collected mosquitoes in the study area. This finding is consistent with that reported by Jupp *et al.*, (2002) [17], in the 2000 outbreak where *Cx. tritaeniorhynchus* and *Ae.v.arabiensis* were incriminated as primary vectors for RVF. Furthermore, the *Culex* abundance rate obtained from this study (94.39%) was similar in rates to that previously reported in Sudan during the 2007 outbreak [18]. Historically, *Aedes* mosquitoes were incriminated as principal vectors for RVF and believed to play a significant role in maintaining the endemicity of the disease in the environment through transovarial transmission [19]. Contrary to the *Culex*, *Aedes* which was found infrequently distributed (22.4%) in our study, reported high abundance rate in Sabia (2.46%), followed by Alardah (02.0%). However, such results highlighted the two districts as high-risk areas for RVF transmission particularly during inter-epizootic periods. Several studies have shown that phlebotomus Sandflies

were involved in vectoring many viruses of great medical and veterinary importance including RVF [20]. Preliminary surveys of sandflies revealed that they were the most likely vectors for both cutaneous and visceral leishmaniasis [21]. Some previous studies indicated that they were more common and abundant in lowlands with respect to higher altitudes. (Al-Zahrani *et al.*, 1997) reported that the dominant species in Jazan were (*Phlebotomus sergenti* Parrot) in the highlands and (*P. bergeroti* Parrot) in the lowlands. In both habitats, there was a marked seasonal variation in abundance, peak levels in the lowlands preceding the highlands [22]. However, the present study indicated that Sandflies were distributed sporadically in altitudes between 24-760 meter above sea level (Figure 5.1). It was found more abundant in Bulgazi, Almsarha and Abuareesh with values (5.6%, 4.36%, 3.89) respectively. Such findings were previously recorded in Asir region where (5.12%) and (1.8%) of flies were collected from Sarroat mountains(1600-3100 meter) and Asir Plateo (1220 to 1750) meter above sea level [23]. However, the introduction of these flies in the region clearly explained the endemicity of cutaneous and visceral leishmaniasis in many areas in Jazan region [24].

Culicoides are known as the major vectors of economically important arboviruses and particularly arboviruses of domestic livestock such as Blue Tongue, African horse Sickness, Bovine ephemeral fever [25]. Studies conducted in Jazan region to investigate the incidence of Blue Tongue disease reported high prevalence in sheep, goats, cattle and camels (65.8%), (68.2%), (49.3%) and (44%) respectively [26]. Therefore, the observed high incidence rate of BT in Jazan could be justified by the distribution of *Culicoides* in most districts particularly in Almsarha (3.13%) and Abuareesh (2.18%). RT-PCR assay is considered as sensitive and specific test for RVF diagnosis by detecting the genome of the virus. Moreover, the results of this study showed that all mosquito samples were found negative for PCR test. Interestingly, molecular investigations demonstrated that, RVFV was circulated in mosquitoes at very low rates even during outbreaks. Likewise, in the 2000 outbreak in Jazan region, only six of 15 428 *Culex tritaenio-rhynchus Giles* and seven of 8091 *Aedes vexans arabiensis* were tested positive for the presence of RVFV [27].

The geospatial analysis estimated that around (24,486) people in Alardah, Aljadiah, Alaedabi and Bulgazi were located within mosquito flight range and more likely to be affected by mosquito bites and risk of arboviral infections (Figure 5.2).

Since little is known about the temporal abundance of mosquito and *midge* species in the surveillance zones, future researches should include monitoring the spatio-temporal distribution of RVF vectors and seasonal variations in abundance and peak level to enhance the current surveillance efforts.

In conclusion, spatio-temporal distribution of mosquitos based on species identification should be considered in future researches towards more effective control measures against mosquito-borne diseases. The study highlighted the cities of Alardah, Aljadiah, Alaedabi and Bulgazi as hot spots of mosquito borne diseases. Consequently, special attention should be given to these areas regarding vector control program, public awareness and self-protective measures.

References

- World Health Organization (WHO) 2016. Rift Valley Fever. Media Centre, fact sheet N207. <http://www.who.int/mediacentre/factsheets/fs207/en/>
- Centers for Disease Control and Prevention CDC, 2013. <https://www.cdc.gov/vhf/rvf/outbreaks/distribution-map.html>
- Daubney R, Hudson JR, Garnham PC. Enzootic hepatitis or Rift Valley fever. An undescribed virus disease of sheep, cattle and man from East Africa, J. Pathol. Bacteriol. 1931; 34:545-579.
- Madani TA, Al-Mazrou YY, Al-Jeffri MH, Mishkhas AA, Al-Rabeah AM, Turkistani AM *et al.* Rift Valley fever epidemic in Saudi Arabia: epidemiological, clinical, and laboratory characteristics. Clin Infect Dis. 2003; 37(8):1084-1092
- Davies FG, Linthicum KJ, James AD. Rainfall and epizootic Rift Valley fever. Bull World Health Organ, 1985; 63(5):941.
- Turell MJ, Dohm DJ, Mores CN, Terracina L, Walette Jr DL, Hribar LJ *et al.* Potential for North American Mosquitoes to Transmit Rift Valley Fever Virus1. J Am Mosq Control Assoc. 2008; 24(4):502-507.
- Rayah E, Amin E, Himeidan YE, Kweka EJ, Mahgoub MM, Ouma JO. Recent Outbreaks of Rift Valley Fever in East Africa and the Middle East, 2016. <http://khartoumspace.uofk.edu/handle/123456789/19336>
- Jupp PG, Kemp A, Grobbelaar A, Leman P, Burt FJ, Alahmed AM *et al.* The 2000 epidemic of Rift Valley fever in Saudi Arabia: mosquito vector studies. Med Vet Entomol, 2002; 16(3):245-252.
- Miller BR, Godsey MS, Crabtree MB, Savage HM, Al-Mazrao Y, Al-Jeffri MH *et al.* Isolation and Genetic Characterization of Rift Valley fever virus from *Aedes vexans arabiensis*, Kingdom. Emerg Infect Dis, 2002; 8(12):1493.
- Emirate of Jazan region (2016). https://www.moi.gov.sa/wps/portal/Home/emirates/jeezan/contents!/ut/p/z1/pVPRboIwFP0VX_a49EILhceOTUAxEw1T-kIAq2JGQWW67OtXjcuYLMiW-9K0Pef03tNTxNEccZkeilXaFJVMX9U65mYCPiGeRvShRcZ9YKE-cJjj64A1NDsD7NB1PE8BXI2ZwHyqP4NvaK6vI_4XvuMyj9AAwApcA3zmRRM7xBgYbuUH-MKHK4NBF_8FccRz2dTNGsWb9COVd3CZ0qx6a3rNWvTqXXUoZC7U0YdMfu8nQp5k6rxYoHgJC5NmFhCCMc1zYeOMLKkpRJpphFrGGbk_nqCcfpfmWhNDITbCdAp97Prw1dp173h757PTTR3mdmwnw_657PEBGMOhPTR8DQKC4vYu1AMfCnFEkax2pcrT9B8-edCITm9Qb5dm-g3Sg67Qq19VbLZbzIT0KtmI9wbN8leXUZRVFq4vJ9DMS6dzBo9YWNVJqM9-wT7o_xR/dz/d5/L2dBISEvZ0FBIS9nQSEh/
- Masood MF. Ecological distribution of Snakes fauna of Jazan region of Saudi Arabia. Egypt. Acad. J. biology. Sci. 2012; 4(1):183-197.
- Means RG. Mosquitoes of New York. Part I. The genus *Aedes* Meigen with identification keys to genera of Culicidae. New York State Museum Bulletin, 1979, 430a.
- Aïssaoui L, Boudjelida H. Diversity and distribution of culicinae fauna in Tebessa district (North-East of Algeria). Inter J Mosq Res. 2017; 4(1):07-12.
- Rydzanicz K, Lonc E. Species composition and seasonal dynamics of mosquito larvae in the Wrocław, Poland area. J Vector Eco. 2003; 28:255-266.
- Gupta R, Shriram R. Disease surveillance and monitoring using GIS. Map India conference, 2004. <http://www.gisdevelopment.net/application/health/planning/pdf/mi04054.pdf>
- Sall AA, Thonnon J, Sene OK, Fall A, Ndiaye M, Badez B *et al.* Single-tube RT-PCR for detection of Rift Valley fever virus in human and animal sera. J. Virol. Methods, 2001; 91:85-92.
- Jupp PG, Kemp A, Grobbelaar A, Leman P, Burt FJ, Alahmed AM *et al.* The 2000 epidemic of Rift Valley fever in Saudi Arabia: mosquito vector studies. Med Vet Entomol, 2002; 16(3):245-252.
- Seufi AM, Galal FH. Role of *Culex* and *Anopheles* mosquito species as potential vectors of rift valley fever virus in Sudan outbreak, 2007. BMC infectious Diseases, 2010; 10(1):65.
- Rayah E, Amin E, Himeidan YE, Kweka EJ, Mahgoub MM, Ouma JO. Recent Outbreaks of Rift Valley Fever in East Africa and the Middle East, 2016. <http://khartoumspace.uofk.edu/handle/123456789/19336>

20. Turell MJ, Perkins PV. Transmission of Rift Valley fever virus by the sand fly, *Phlebotomus duboscqi* (Diptera: Psychodidae). *Am J Trop Med Hygen*, 1990; 42(2):185-188.
21. Morris RV, Shoemaker CB, David JR, Lanzaro GC, Titus RG. Sandfly maxadilan exacerbates infection with *Leishmania major* and vaccinating against it protects against *L. major* infection. *J Immunol*, 2001; 167(9):5226-5230.
22. Al-Zahrani MA, Lane RP, Chin IC, Asiry MA, Peters W. Biology of Phlebotomus sandflies (Diptera: Psychodidae) in two contrasting leishmaniasis foci of south-west Saudi Arabia. *Bull Entomol Res*, 1997; 87(3):221-230.
23. Ibrahim AA, Abdoon MA. Distribution and Population Dynamics of Phlebotomus Sandflies (Diptera, Psychodidae) in an Endemic Area of Cutaneous leishmaniasis in Asir Region, Southwestern Saudi Arabia. *J Entomol*. 2005; 2(1):102-108.
24. Al-Jaser MH. Studies on the epidemiology of malaria and visceral leishmaniasis in Jizan area, Saudi Arabia. *J King Saud Univ. Sci*, 2006; 19:9-19.
25. Mellor PS, Boorman J, Baylis M. Culicoides biting midges: their role as arbovirus vectors. *Annul Rev Entomol*, 2000; 45(1):307-340.
26. Yousef MR, Al-Eesa AA, Al-Blawi MH. High seroprevalence of bluetongue virus antibodies in Sheep, Goats, Cattle and Camel in different districts of Saudi Arabia. *Vet World*, 2012; 5(7).
27. Jupp PG, Kemp A, Grobbelaar A, Leman P, Burt FJ, Alahmed AM *et al*. The 2000 epidemic of Rift Valley fever in Saudi Arabia: mosquito vector studies. *Med Vet Entomol*, 2002; 16(3):245-252.