



## Original article

# First record of West Nile Virus detection inside wild mosquitoes in Khartoum capital of Sudan using PCR



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## ABSTRACT

This study aimed to explore the presence of West Nile Virus (WNV) inside four species of mosquitoes: *Culex univittatus* (Theobald), *Culex quinquefasciatus* (Say), *Aedes vittatus* (Bigot) and *Aedes vexans* (Meigen). Adult wild mosquitoes were collected from different sites: Soba West, Hellat Kuku, Shambat, and Khartoum North Central Live Stock Market (KCLM). Surveys were carried out at Khartoum State during two phases: pre to the rainy season and post to the rainy season. Mosquito specimens were identified using classical keys then preserved at  $-80^{\circ}\text{C}$  freezer for two weeks till the virus examination using polymerase chain reaction (PCR) was carried out. WNV has been detected inside the three species of mosquitoes: *A. vexans*, *C. univittatus*, and *C. quinquefasciatus*. The species were collected from Hellat Kuku, (Shambat and Hellat Kuku), and (Shambat and KCLM) respectively. Two species of mosquitoes were positive for the virus: *C. quinquefasciatus* and *C. univittatus*. Positive results for the virus during the first phase of the study; males of *C. quinquefasciatus* and *C. univittatus* collected during the second phase of the study were also tested for the existence of the virus and they were positive. For our knowledge this study represents first record of WNV inside wild mosquitoes in Sudan. PCR technique provided reliable information because specific primer-probe sets were used for the detection of the virus. Extra studies are required to incriminate these species of mosquitoes as potential vectors of WNV.

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## 1. Introduction

Viruses transmitted by arthropods cause many diseases to humans. Many mosquitoes transmit viral infections even they may have a negative impact for both man and animals such as

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Dengue fever/Dengue haemorrhagic fever (DF/DHF), Rift Valley Fever (RVF), West Nile Viruses (WNV), and Yellow Fever (Rigau-Pérez et al., 1998, Lanciotti et al., 1999, Soliman et al., 2010). West Nile Virus has first been discovered in 1999 in the USA and soon disseminated to nearby countries such as Central America and Canada (Hollidge et al., 2010, Weissenböck et al., 2010, Chaskopoulou et al., 2013). Recently, WNV is one of the most globally distributed and it is endemic in many developed as well developing countries throughout the Continents (Lanciotti et al., 1999). West Nile Virus (WNV) is a Flavivirus contains a single positive strand of RNA (Khromykh et al., 2001, Friebe and Harris, 2010, Londono-Renteria and Colpitts, 2016). The most harmful morbidities of WNV disease is serious encephalitis in horses and humans, besides the infection may be fatal in some domestic and wild birds. It is a Zoonotic disease emerges and resurges as an authenticity trouble due to weather and societal modifications. West Nile Virus is transmitted by mosquitoes. In about 75% of cases patients have

few or do not develop any symptoms (Petersen et al., 2013). Around 20% of patients have febrile chills, headache, spew, or skin eruption. Presentations of sharp sickness comprise high febrile episodes, headache, neck rigidity, confusion, locomotion disorders coma, shiver, convulsions, muscle ache, sight loss, anesthesia and palsy (Prevention, 2018).

West Nile Virus neuro-invasive disease (WNNV) is comprised with the loss of hippocampal synapses with lack of cure. Adult neurogenesis and synaptogenesis are essential features of hippocampal repair, which suggests that viruses affect these processes as well, can cause death in adults (Öncü et al., 2018). WNV is found naturally circulating between birds, mosquitoes and other mammals like humans and horses. WNV pathogen affect human's brain causing encephalitis (Lanciotti et al., 1999, Lanciotti et al., 2000). Suburban conditions seem adequate for propagation cycles of the transmission of the virus to mammalian hosts (Wagner et al., 2018).

WNV initially discovered in 1937 from a blood sample of a female (had a fever) inhabiting the West Nile Province of Uganda then the virus was detected in Egypt in 1950s in mosquitoes, birds as well as other human patients. The virus was not detected since that time in Africa until the years 1974 and 1983–1984 when intermittent outbreaks in South Africa were registered. Within the last fourteen years, human epidemics occurred in Africa: Algeria; 1994, Tunisia; 1997, and Congo; 1998 (Burakoff et al., 2018).

In neoteric years, devastating outbreaks of the disease have been reported from many countries (Depoortere et al., 2004, Lindsey, Lehman et al., 2015, Krow-Lucal et al., 2017). The recent documented outbreak of WNV occurred in Ngorban County, South Kordofan province, Sudan 2002 (Grubaugh et al., 2017), but the first information on WNV outbreak in Sudan obtained from the Nuba Mountains in the same province in 1956 where WNV antibodies were documented in children aged up to 14 years in the Nuba Mountains (Watts et al., 1994). WNV outbreaks happened in the Northern State of Sudan in 1989 and in Nuba mountains in 2002 (Toschi, 1954, Watts et al., 1994). In Sudan, WNV infections have observed mainly among kids and demonstrated as encephalitis including severe neuro-complications. The spread of such a virus to other countries cannot be excluded (Grubaugh et al., 2017). WNV oversight differs between the countries of different continents (Strausbaugh et al., 2001, Abdelgadir et al., 2010), extending from clinical examination of horses, humans, birds or other infected animals through typical inspection and the detection of the virus inside mosquitoes vectors. Horses can be diagnosed for WNV before humans due to their higher sensitivity to the virus compared to humans (Hollidge et al., 2010, Weissenböck et al., 2010). The advanced isolation or detection of the virus in mosquitoes may be significantly helpful for a fast and precise diagnosis.

Hypothesis of this study depended on a previous study which recorded at least three of the four mosquitoes' species involved in this study are established at Khartoum State and found to be harboring some arboviruses inside them such as Rift valley Fever virus when examined by PCR technique (Mohamed et al., 2004, Abdelgadir et al., 2010). This gave the rationale to screen the virus inside four species of mosquitoes: *Cx. univittatus*, *Cx. quinquefasciatus* *Ae. vexans*, and *Ae. vittatus* using PCR.

## 2. Materials and methods

### 2.1. Study area

The State of Khartoum is located between 15 10" and 16 30" N latitude and 31 35" and 40 20" E longitude. Four sites in Khartoum State (Soba West, Hellat Kuku, Shambat, KCLM) were selected for

this study depending on certain risk factors such as the high density of mosquitoes. Certain surveys were conducted in these sites post to the rainy season (between October 2016 to January 2017) and pre to the rainy season between (April – July 2017).

### 2.2. Ethical approval

The study was approved by the ethical board of National Ministry of health- Sudan. Participants who agreed to allow the researchers to collect mosquitoes from inside and around their houses in the four study sites were asked to sign informed consent forms before the beginning of the study.

### 2.3. Sample collection

Adult males and blood fed females of the targeted mosquito species were collected by aspirators from outdoors resting sites such as earthen jars 'zeers', septic tanks and wells. Indophilic mosquitoes were aspirated from inside rooms. Collected specimens were identified using morphological keys (Hopkins, 1952) and categorized morphologically (Fig. 1), placed into labeled tubes then transported in cool containers to the laboratory. Mosquito specimens were anesthetized using triethyl amine then re-classified under dissecting microscopes using classical keys (Strausbaugh et al., 2001). Identified specimens collected from each study site were then separated into labeled cryovials and preserved into –80 °C freezer. At the end, a total of 2690 samples (separated into ten pools) have been examined for WNV using RT-PCR method. The sample size has been chosen as recommended before (Mohamed et al., 2004, Abdelgadir et al., 2010).

### 2.4. Entomological study

Mosquito pools were homogenized by shaking with 3 mm tungsten carbide beads (QIAGEN, Hilden, Germany) in 500–600 µl of Eagle's least essential medium, added to 5% fetal bovine plasma 1% penicillin–streptomycin and 1% L-glutamine. They were purified by centrifugation at 4,000 × rpm for 4 min, divided into tubes and preserved at –80 °C. Nucleic acids were clarified from one aliquot of each pool using High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Mannheim, Germany), Then reverse transcription polymerase chain reaction conducted (TaqMan® one-step RT-PCR master mix reagents kit). The One-Step EZ RT-PCR TaqMan® reagent kit (Applied Biosystems, Sweden) reaction performed according to the manufacturers' guidelines (Jupp et al., 2002). All collected samples were classified into their species using available morphological keys (The ten mosquito pools were grinded in a varying volume of Virus Transport Media (VTM) depending on the pool size according the method of (Zakhia et al., 2018). The supernatants were clarified through a 3.5 µm filter and centrifuged at 3000 cycle/30 min.

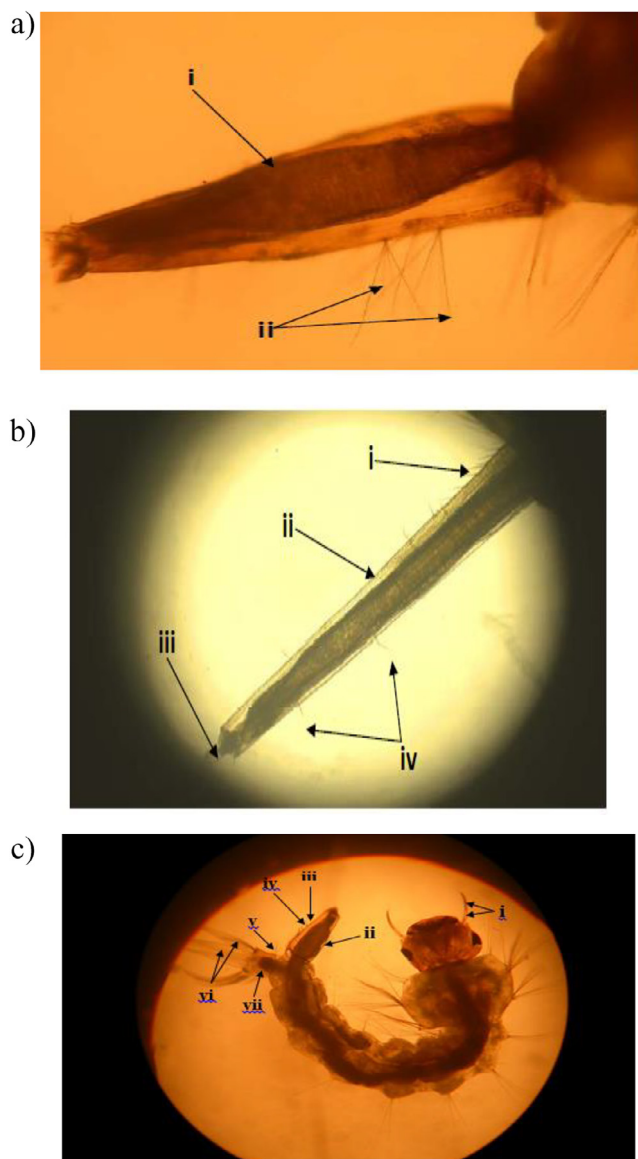
### 2.5. RT-PCR

In this study researchers concentrated on primer styling for disclosure of WNV inside mosquitoes. RNA was extracted from the purified samples where RNA was washed in 40 µl washing buffer used in the RT-PCR reaction. The series of WNV primer used in this research was as follows:

Forward 5' -CAGACCACGCTACGGCG- 3' (Nucleotides 10,668–10,684).

Reverse 5' -CTAGGGCCGCTGGG- 3' (nucleotides 10,770–10,756), and Probe 5' - (6-Fam) TCTGCCGAGAGTGCAGTCTGCCAT (BHQ 1 ~ Q)- 3' (nucleotides 10,691 – 10,714).

For each RNA sample the following reagents were added (X one reaction) to a thin walled 0.2 ml PCR tube: RNA template (2 ng- 2 µg), 1.0 µl of the RT primer (Ranomnonamer primer), and



**Fig. 1.** Morphological characterization of the mosquitos investigated during the current study (a) Characteristic features of *Culex quinquefasciatus* 4th instar larvae i- Siphon with markedly convex sides. Siphon index usually more than 3. ii- Sub ventral tufts numerous. (b) Characteristic features of 4th instar larvae of *Culex univittatus* i- Pectin spines toothed ii- Siphonal index above 6 iii- Middle spin between valvesiv- Ventral tufts numerous restricted to the distal two thirds. (c) Characteristic features of 4th Instar Larvae of *Aedes vexans* mosquitoes. i. Spines of antennae widely spaced, antennae shorter than head. ii. Siphon short and stout. iii. Single sub ventral tuft. iv. Distal siphon spines detached. v. Caudal setae numerous. vi. Gills are relatively short. vii. Saddle incompletely fills the caudal segment.

RNAse/DNAse free water (4.0  $\mu$ l). The final volume was 10  $\mu$ l. A lid has been applied to each sample then samples were heated to 65  $^{\circ}$ C for 5 min using a heater water bath then the tubes soon cooled in snowy water bath. After that a mix was prepared as follows (per one reaction):

qScript 10X Buffer (2.0  $\mu$ l), dNTP mix 10 mM of each (1.0  $\mu$ l), DTT 100 mM (2.0  $\mu$ l), RNAse/DNAse free water (4.0  $\mu$ l), qScript enzyme (1.0  $\mu$ l), the final volume was 10  $\mu$ l. Aliquots of the PCR products (10 ml) were electrophoresed on 1.2% agarose gel in Tris-borate buffer and ethidium bromide and DNA bands were visualized on a UV trans illuminator. The positive samples were used for RT-PCR investigation in order to obtain the amplification curves.

## 2.6. Statistical analysis

Results were statistically analyzed using the Anderson–Darling test, as well as for homogeneity variances. The data was normally distributed and is expressed as the mean  $\pm$  standard error of the mean (SEM). Significant differences among the groups were analyzed by one- or two-way ANOVA followed by Tukey's post-test using SPSS software, version 17. Variations were deemed statistically significant at  $P < 0.05$ .

## 3. Results

### 3.1. Seasonal variation of mosquitos and arboviruses surveillance in four different regions of Khartoum State during post rainy seasons

During the post-rainy season, different specimens from four localities in Khartoum State were investigated. The four localities are Soba West, Shambat, Live Stock Market and Hellat Kuku. Fig. 2 illustrates the electronic map of these collection sites. Overall the most abundant mosquito type was *Culex quinquefasciatus* (54.5%), which was the dominant species in all of the investigated regions (Table 1). *Aedes vexans* was the second prevalent mosquito type (29.9%), was found in both localities of Soba West and Hellat Kuku. Other mosquito species which have no direct role in transmitting WNV, were not included in the current study. Positive results for WNV were detected in pools of *Culex quinquefasciatus*, *Culex univittatus* and *Aedes vexans* in Shambat, live stock market and Hellat Kuku (Table 1).

### 3.2. Seasonal variation of mosquitos and arboviruses surveillance in four different regions of Khartoum State during pre rainy seasons

During the pre-rainy season, the dominant mosquito species were *Culex quinquefasciatus* and *Culex univittatus*. While *Culex quinquefasciatus* was prevalent in all the four investigated regions, *Culex univittatus* was prevalent only in shambat region (Table 2). Positive results for WNV were detected in pools of *Culex quinquefasciatus* and *Culex univittatus* in Shambat and live stock market. Conversely, in both of Soba West and Hellat Kuku, no viral infections could be detected (Table 2).

### 3.3. PCR analysis for the positive pools samples

As showed in Fig. 3a, the plot of cycle numbers against fluorescence signals (amplification plot) which correlates with the initial amount of target DNA during the exponential phase of PCR, has revealed different patterns. In Fig. 3b, when the threshold cycle (CT) was plotted against the concentration (standard curve), it was clear the reverse relationship between the concentration and the CT values.

### 3.4. Agarose gel electrophoresis for the DNA of the WNV inside three pools of mosquitos during the post rainy season

PCR products were electrophoresed on 1.2% agarose gel in Tris-borate buffer and ethidium bromide and DNA bands were conceived on a UV trans illuminator. Fig. 4 illustrates the results of the gel electrophoresis whereas the first lane represents the molecular marker (the ladder), lanes two and nine represents the positive controls, lane three is the negative control. Negative samples are represented in lanes five, seven, eight, twelve and thirteen while the positive samples are represented in lanes four, six, ten, eleven and fifteen.

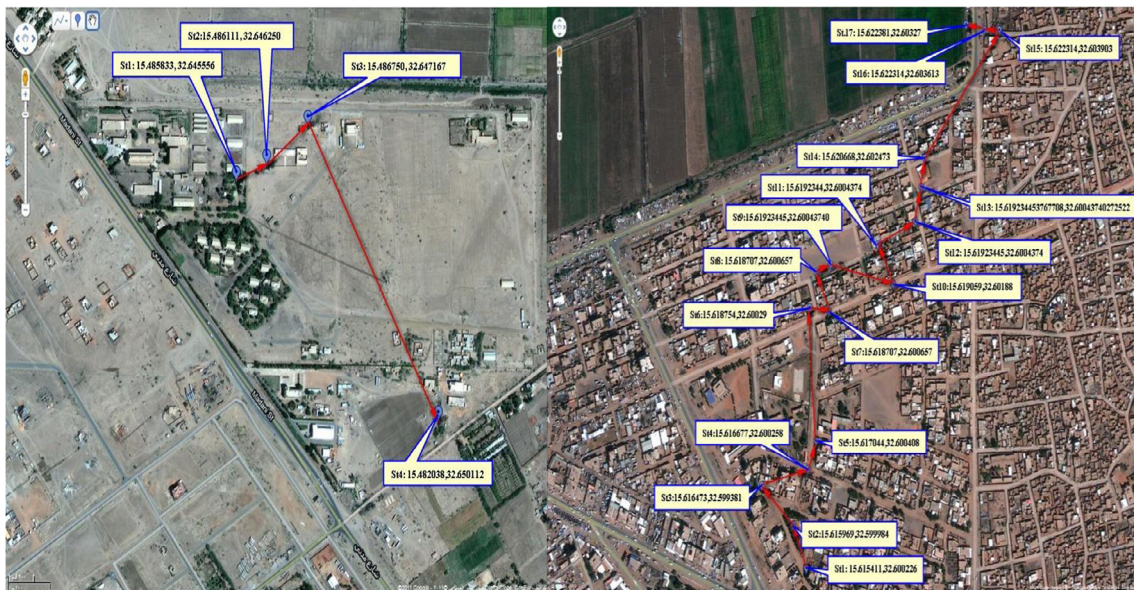


Fig. 2. Electronic Map showing collection sites of most WNV-positive mosquitoes pools (Soba West). Locations on the map show altitude and longitude of each station.

Table 1  
Arboviruses surveillance during the 1st season of the study (Post Rainy Season).

Number of pool	Species	Study Site	Number of specimens/pool	Positive pools
1	<i>Aedes vexans</i>	Soba West	300	–
2	<i>Culex univittatus</i>	Soba West	300	–
3	<i>Culex quinquefasciatus</i>	Soba West	300	–
4	<i>Culex quinquefasciatus</i>	Shambat	300	Positive
5	<i>Culex univittatus</i>	Shambat	300	Positive
6	<i>Culex quinquefasciatus</i>	Live Stock Market	300	Positive
7	<i>Culex univittatus</i>	Live Stock Market	286	–
8	<i>Culex quinquefasciatus</i>	Hellat Kuku	300	–
9	<i>Culex univittatus</i>	Hellat Kuku	300	Positive
10	<i>Aedes vexans</i>	Hellat Kuku	4	Positive

Table 2  
Arboviruses surveillance during the 2nd season of the study (pre- rainy season).

No. pool	Species	Study site	No. specimens/pool	Positive pools
1	<i>Culex quinquefasciatus</i>	Soba West	300	–
2	<i>Culex univittatus</i>	Shambat	72	Positive
3	<i>Culex univittatus</i>	Shambat	145	Positive
4	<i>Culex quinquefasciatus</i>	Shambat	300	Positive
5	<i>Culex quinquefasciatus</i>	Shambat	300	Positive
6	<i>Culex quinquefasciatus</i>	Live Stock Market	245	Positive
7	<i>Culex quinquefasciatus</i>	Hellat Kuku	300	–

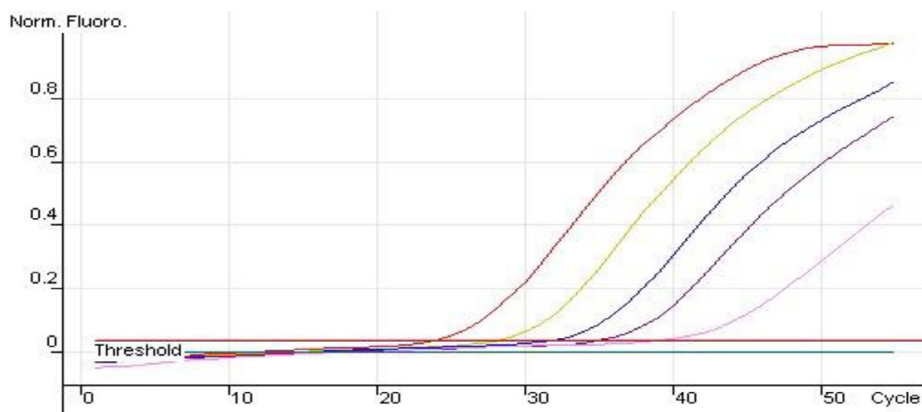


Fig. 3a. Amplification curve for the positive pools samples.

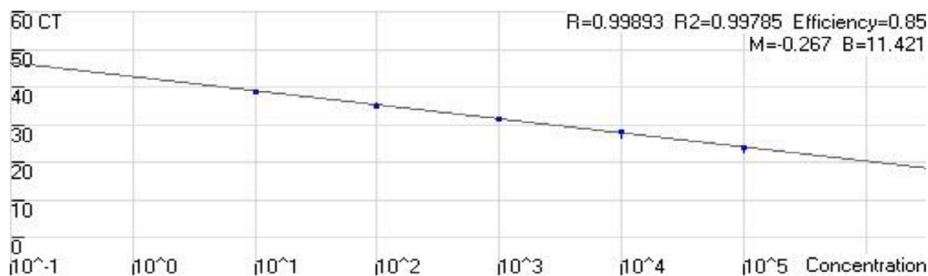


Fig. 3b. Standard curve for the positive pools samples.

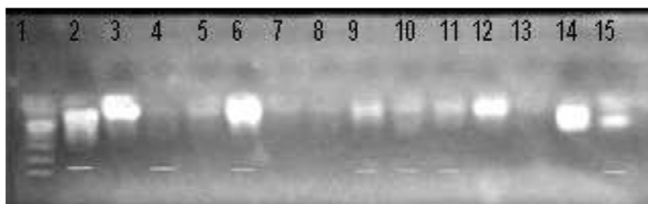


Fig. 4. Conventional PCR for the detection of WNV virus inside three pools of mosquitoes collected during the 1st phase of the study. Lane 1 Molecular Marker, Lane 2 and 9 Positive Control, Lane 3 Negative Control (70 bp), Lane 5, 7, 8, 12, and 13 Negative Sample. Lane 4, 6, 10, 11 and 15 Positive samples (70 bp).

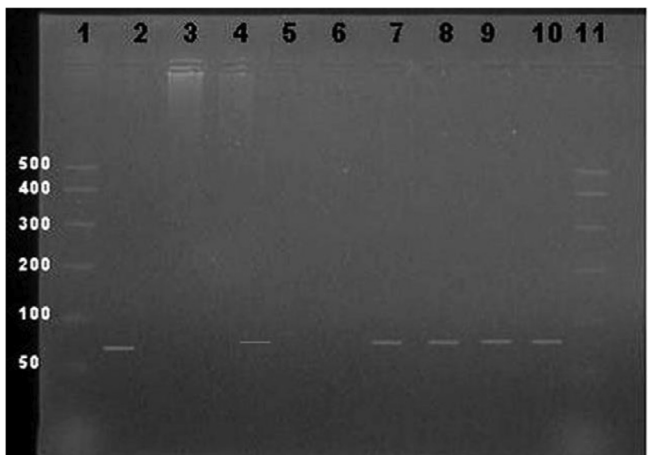


Fig. 5. Conventional PCR for the detection of WNV virus inside pools of mosquitoes collected during the 2nd season of the study. Lane 1 & 11 Molecular Marker, Lane 2 Positive Control (70 bp), Lane 3 Negative Control, Lane 5 and 6 Negative Sample, Lane 4, 7, 8, 9 and 10 Positive samples (70 bp).

### 3.5. Agarose gel electrophoresis for the DNA of the WNV inside three pools of mosquitos during the pre-rainy season

PCR products were electrophoresed on 1.2% agarose gel in Tris-borate buffer and ethidium bromide and DNA bands were seen on a UV trans illuminator. Fig. 5 illustrates the results of the gel electrophoresis whereas the first and the last lanes represents the molecular marker (the ladder), lanes two and lane three represents the positive and negative controls respectively. Negative samples are represented in lanes five and six while the positive samples are represented in lanes four, seven, eight, nine and ten.

## 4. Discussion

Vector transmitted viruses (arboviruses), like yellow fever and Dengue fever viruses, chikungunya virus, and West Nile Virus (WNV) cause persistent impendence to protrude and give rise

serious epidemics (Grubaugh et al., 2017). It is well known that WNV is embroiled in an enzootic cycle including birds and mosquitoes in the genus *Culex* (Hopkins, 1952, Watts et al., 1994). A previous study mentioned that WNV is an excellent example of an introduced RNA virus that adapted to a new environment (i.e., new genotype linked to a shorter extrinsic incubation period in local mosquitoes), promoting its spread throughout the world (Strausbaugh et al., 2001). In Sudan some study investigated the sero-prevalence of WNF among suspected patients. A recent study investigated focused on WNV and Dengue. This study demonstrated that IgG antibodies were found to be 64% for WNV and 14% for DFV (Mohamed et al., 2004). Limited data is available about the circulation of the virus among mosquito vectors using PCR technique for detection of the virus. This gave the rationale of our research which endeavoured to explore the existence of WNV inside four species of mosquitoes, namely: *Cx. univittatus* (Theobald), *Cx. quinquefasciatus*, *Ae. vexans* (Meigen), and *Ae. vittatus* (Bigot). The study sites have been selected due to the detection of a Bunya virus inside wild collected from these in a previous study (Mohamed et al., 2004). WNV has been detected inside three species of mosquitoes: *Ae. vexans*, *Cx. univittatus*, and *Cx. quinquefasciatus* collected before the rainy season. These species were collected from Hellat Kuku, (Shambat and Hellat Kuku), and (Shambat and KCLM) respectively. To insure the results, we collected wild mosquitoes from the same locations after the rainy season (phase 2 of the study). All samples collected during the second phase have also been screened for the virus. Only two species of mosquitoes were positive for the virus: *Cx. quinquefasciatus* and *Cx. univittatus* while *Ae. vexans* were absent from the collection sites after the rainy season. They assumed to be diapaused waiting for the rainy season next year (Jupp et al., 2002). The first positive mosquito species has been collected from Shambat and KCLM while the second positive species has been collected from Shambat. Since found positive for the virus during phase one of the study; males of *Cx. quinquefasciatus* and *Cx. univittatus* collected during the second phase of the study were also tested for the existence of the virus and they were both affirmative. For our knowledge this is the first record of WNV detection inside wild mosquitoes in Sudan. These results coincide with finding of a study demonstrated experimental transmission of WNV by *Culex pipiens* from Lebanon (Depoortere et al., 2004). In Sudan; a study conducted after WNV outbreak which happened in Ngorban County, South Kordophan, Sudan during the year 2002 (Abdelhalim and Kafi, 2014, RE, 2014). This study explored the outbreaks and performed a case-control study in the hamlet of Limon. The results showed laboratory analysis assured existence of WNV IgM antibodies in eight out of thirteen cases. Another study determined the propagation of IgG and IgM in many blood transfused patients in Khartoum State, Sudan (Organization, 2017). Results of these studies revealed that fifty patients out of ninety one (54%) were affirmative for WNV IgG antibodies whilst 11 patients constituting 12.1% were positive for WNV IgM antibodies. In 2014, the Sudanese Ministry of Health (SMOH) and the World Health Organization

(WHO) notified an outbreak of viral hemorrhagic fever (VHF) in Darfur, Sudan. According to the SMOH, 182 dubitable VHF cases, involving 103 passed cases, were recorded in 12 regions in South, East, Central, West and North Darfur. Around 36 specimens were collected from dubitable cases and their co-patients. Eight samples out of these 36 specimens (collected from Central, West and North Darfur) were confirmed for West Nile Virus (RE, 2014, Organization, 2017).

## 5. Conclusions

Results of this study achieved the objective of WNV detection inside three species of mosquitoes using PCR. No available data was available regarding investigation of WNV inside mosquito vectors so our study is thought to open a door for further investigation for the competence of these species of mosquitoes in the transmission of the virus. Detection of the virus in these mosquito species requires more studies to incriminate their role in WNV. Subsequently; more studies are essential to define the part of mosquitoes in human to human transition of the virus.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## References

Abdelgadir, D.M., Bashab, H.M., Elhadi Mohamed, R.A., Abuelmaali, S.A., 2010. Risk factor analysis for outbreak of rift valley fever in Khartoum state of Sudan. *J. Entomological Sci.* 45 (3), 239–251.

Abdelhalim, K.A., Kafi, S.K., 2014. Seroprevalence of West Nile Fever & Dengue Fever Viruses in Suburban areas in Khartoum State, Sudan. *Am. J. Res. Commun.* 2 (8), 81–86.

Burakoff, A., Lehman, J., Fischer, M., Staples, J.E., Lindsey, N.P., 2018. West Nile virus and other nationally notifiable arboviral diseases—United States, 2016. *Morb. Mortal. Wkly Rep.* 67 (1), 13.

Chaskopoulou, A., Dovas, C.I., Chaintoutis, S.C., Kashefi, J., Koehler, P., Papanastassopoulou, M., 2013. Detection and early warning of West Nile Virus circulation in Central Macedonia, Greece, using sentinel chickens and mosquitoes. *Vector-Borne and Zoonotic Diseases* 13 (10), 723–732.

Depoortere, E., Kavle, J., Keus, K., Zeller, H., Murri, S., Legros, D., 2004. Outbreak of West Nile virus causing severe neurological involvement in children, Nuba Mountains, Sudan, 2002. *Trop. Med. Int. Health* 9 (6), 730–736.

Friebe, P., Harris, E., 2010. Interplay of RNA elements in the dengue virus 5' and 3' ends required for viral RNA replication. *J. Virol.* 84 (12), 6103–6118.

Grubbaugh, N.D., Fauver, J.R., Rückert, C., Weger-Lucarelli, J., Garcia-Luna, S., Murrieta, R.A., Gendernalik, A., Smith, D.R., Brackney, D.E., Ebel, G.D., 2017. Mosquitoes transmit unique West Nile virus populations during each feeding episode. *Cell Rep.* 19 (4), 709–718.

Hollidge, B.S., González-Scarano, F., Soldan, S.S., 2010. Arboviral encephalitides: transmission, emergence, and pathogenesis. *J. Neuroimmune Pharmacol.* 5 (3), 428–442.

Hopkins, G.H.E., 1952. Mosquitoes of the Ethiopian Region. I. Larval Bionomics of Mosquitoes and Taxonomy of Culicine Larvae. Mosquitoes of the Ethiopian Region. I. Larval Bionomics of Mosquitoes and Taxonomy of Culicine Larvae. (Edn 2)

Jupp, P., Kemp, A., Grobbelaar, A., Leman, P., Burt, F., Alahmed, A., Mujalli, D.A., Khamees, M.A., Swanepoel, R., 2002. The 2000 epidemic of Rift Valley fever in Saudi Arabia: mosquito vector studies. *Med. Vet. Entomol.* 16 (3), 245–252.

Khromykh, A.A., Meka, H., Guyatt, K.J., Westaway, E.G., 2001. Essential role of cyclization sequences in flavivirus RNA replication. *J. Virol.* 75 (14), 6719–6728.

Krow-Lucal, E., Lindsey, N.P., Lehman, J., Fischer, M., Staples, J.E., 2017. West Nile virus and other nationally notifiable arboviral diseases—United States, 2015. *MMWR Morb. Mortal. Wkly Rep.* 66 (2), 51.

Lanciotti, R., Roehrig, J., Deubel, V., Smith, J., Parker, M., Steele, K., Crise, B., Volpe, K., Crabtree, M., Scherret, J., 1999. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science* 286 (5448), 2333–2337.

Lanciotti, R.S., Kerst, A.J., Nasci, R.S., Godsey, M.S., Mitchell, C.J., Savage, H.M., Komar, N., Panella, N.A., Allen, B.C., Volpe, K.E., 2000. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. *J. Clin. Microbiol.* 38(11), 4066–4071.

Lindsey, N.P., Lehman, J.A., Staples, J.E., Fischer, M., 2015. West Nile virus and other nationally notifiable arboviral diseases—United States, 2014. *Morbidity and Mortality Weekly Report.* 64(34), 929–934.

Londono-Renteria, B., Colpitts, T.M., 2016. A Brief Review of West Nile Virus Biology. Springer, West Nile Virus, pp. 1–13.

Mohamed, N., Elfaitouri, A., Fohlman, J., Friman, G., Blomberg, J., 2004. A sensitive and quantitative single-tube real-time reverse transcriptase-PCR for detection of enteroviral RNA. *J. Clin. Virol.* 30 (2), 150–156.

Öncü, C., Brinkmann, A., Günay, F., Kar, S., Öter, K., Sarıkaya, Y., Nitsche, A., Linton, Y.-M., Alten, B., Ergünay, K., 2018. West Nile virus, Anopheles flavivirus, a novel flavivirus as well as Merida-like rhabdovirus Turkey in field-collected mosquitoes from Thrace and Anatolia. *Infection, Genetics and Evol.* 57, 36–45.

Organization, W. H., 2017. Sudan health profile 2015, World Health Organization. Regional Office for the Eastern Mediterranean.

Petersen, L.R., Brault, A.C., Nasci, R.S., 2013. West Nile virus: review of the literature. *JAMA* 310 (3), 308–315.

Prevention, C. f. D. C. a., 2018. CDC-West Nile Virus Home.

Rigau-Pérez, J.G., Clark, G.G., Gubler, D.J., Reiter, P., Sanders, E.J., Vorndam, A.V., 1998. Dengue and dengue haemorrhagic fever. *The Lancet* 352 (9132), 971–977.

Soliman, A., Mohareb, E., Salman, D., Saad, M., Salama, S., Fayed, C., Hanafi, H., Medhat, I., Labib, E., Rakha, M., 2010. Studies on West Nile virus infection in Egypt. *J. Infect. Public Health* 3 (2), 54–59.

Strausbaugh, L.J., Marfin, A.A., Gubler, D.J., 2001. West Nile encephalitis: an emerging disease in the United States. *Clin. Infect. Dis.* 33 (10), 1713–1719.

Re, O.C., 2014. Prevalence of West Nile Virus (WNV) AM KHARTOUM.

Wagner, S., Guidi, V., Torgerson, P.R., Mathis, A., Schaffner, F., 2018. Diversity and seasonal abundances of mosquitoes at potential arboviral transmission sites in two different climate zones in Switzerland. *Med. Vet. Entomol.*

Watts, D., El-Tigani, A., Botros, B., Salib, A., Olson, J., 1994. Arthropod-borne viral infectious associated with a fever outbreak in the Northern Province of Sudan. Naval Medical Research Unit No 3 Cairo (Egypt) Dept of Medical Zoology.

Weissenböck, H., Hubálek, Z., Bakonyi, T., Nowotny, N., 2010. Zoonotic mosquito-borne flaviviruses: worldwide presence of agents with proven pathogenicity and potential candidates of future emerging diseases. *Vet. Microbiol.* 140 (3–4), 271–280.

Zakhia, R., Mousson, L., Vazeille, M., Haddad, N., Failloux, A.-B., 2018. Experimental transmission of West Nile Virus and Rift Valley Fever Virus by *Culex pipiens* from Lebanon. *PLoS Negl. Trop. Dis.* 12 (1), e0005983.

## Further reading

Alten, B., Ergünay, K., 2018. West Nile virus, Anopheles flavivirus, a novel flavivirus as well as Merida-like rhabdovirus Turkey in field-collected mosquitoes from Thrace and Anatolia. *Infect., Genetics Evol.* 57, 36–45.