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Seroprevalence and molecular characterization of West Nile Virus in Egypt

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ABSTRACT

West Nile Virus (WNV) is a flavivirus, mosquito-borne infection and have public health importance worldwide. WNV infection have highly significant impact on animal and human health. The virus has been detected serologically in Egypt among equids. Therefore, the aim of the present study to investigate the serological situation of WNV among horse in north of Egypt and identification of WNV in vector. The serological survey was conducted on 500 serum samples that collected from horses from four governorates at north of Egypt. The infection rate was non-significant differed between four localities and the highest rate was reported in Qalyubia governorate (25.5 %) in comparison with other areas. Moreover, the WNV RNA was detected in mosquitoes and the obtained WNV sequence showed high similarity with Eg101 strain and characterized as lineage 1. The obtained findings confirm the circulation of WNV in mosquitoes and animals in Egypt.

1. Introduction

West Nile Virus (WNV) is vector-borne infectious virus belong to genus Flavivirus of family Flaviviridae. The virus is transmitted by mosquitoes belonging to genus Culex [1].

The viral infection circulating between mosquitoes and avian host but have probability to transmitted to human, horses and other mammals. In addition, Horses, human and other mammals considered as dead-end or incidental host but birds serve as natural host for WNV [2–5].

Phylogenetic studies of WNV revealed that presence at least eight evolutionary lineages. Lineages 1 and 2 have been mostly associated with human outbreaks [2]. Additionally, the lineage 3 was identified in the Czech Republic [6], lineage 4 was isolated from Russia [7], lineage 5 was detected in India [8]. Moreover, lineage 6 was described based on small gene fragment from Spain [9] and lineage 7 was recently detected in ticks and rodents in Senegal [10]

WNV is widely distributed and have been reported in numerous countries in Europe, West Asia, Africa and Middle East [1,11,12]. Moreover, the presence of WNV infection has been reported in neighboring countries (Iraq, Israel, Lebanon and United Arab Emirates) of Egypt in mosquitoes and mammalian species [13–15].

In Egypt, WNV was first identified in human and mosquitoes in 1950 followed by several outbreaks during 1952–1954 [16]. Moreover, the serological evidence of WNV infection based on detection of antibodies which was reported in 3% of school children of 8–9 age in Egypt during 1989 [17]. Recently, the WNV antibodies were detected by 20.7 % and 12.7 % in horse and donkey at some localities in the north of Egypt [3], which approved that the WNV circulating in Egypt. In addition, the ecosystem like Nile Delta, flood plains and migratory bird routes are mainly the adequate focal point of infection for WNV.

The previous studies confirmed the presence of WNV antibodies but the identification and characterization of WNV RNA has been failed to date. Therefore, the aim of this study to determine the prevalence of WNV infection in horse and genetic characterization for WNV in mosquitoes.

2. Material and methods

2.1. Ethic statement

This study was carried out according to the principles of good clinical practice and was accepted by the ethical committee for Animal Experiment of Benha University

2.2. Study area

The present study was performed in four governorates (Qalyubia, Menofia, Kafr Elsheikh and Gharbia) at north of Egypt. These governorates are located at 30°25 N to 31°13 E, 30.52 °N 30.99 °E, 38°18 N to 30°56 E and 30.867 °N 31.028 °E (Fig. 1). These governorates were selected according to high density of mosquito population as well as geographical position. These governorates are located in Nile delta which is agricultural area, have high density of mosquitoes and have

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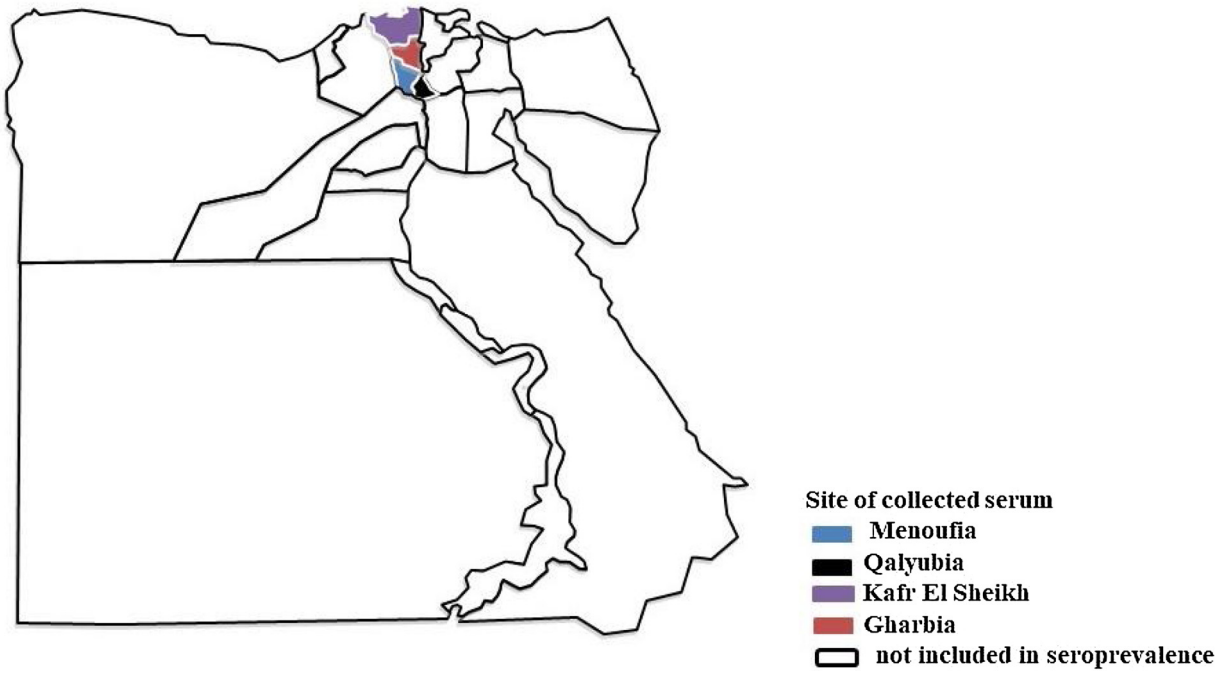

Fig. 1. Map of governorates targeted for sampling in the study.

Table 1
Prevalence of West Nile Fever among horse in some governorates in Egypt.

location	No of examined horse	No of positive	95 % CI	P value
Qalyubia	90	23 (25.5 %)	17.21 – 36.03	0.1
Menofia	120	21 (17.5 %)	14.4 – 25.74	
Kafr Elsheikh	160	34 (21.25 %)	15.35 – 28.56	
Gharbia	130	19 (14.6 %)	9.25 – 22.14	
Total	500	97 (19.4 %)	16.08 – 23.2	

previous serological reports for antibodies against WNV. In addition, it situated in routes of migratory birds which considered as main reservoir for WNV.

2.3. Samples collection

The sample size was estimated using Win Episcope 2.0 (www.winepi.net) with an expected prevalence 10 % and an expected error 5%. The calculated sample size was 250 but we examined 500 serum samples to have a represented samples from the examined localities.

A total 500 serum samples were collected from horses reared by individual farmers that located in four governates at north of Egypt during 2019. The blood samples were collected from examined horse using vacuum tube. The sera were separated from clotted blood by centrifugation at 10,000 xg for 10 min and preserved at −20 °C until exanimation.

Mosquitoes field samples were collected from four equine herds in Kafr Elsheikh and Gharbia governorates using mosquito light trap. The light trap was located at 1 m above the ground, the trap placed in the farm one night per week from 6:00 PM to 8:00 AM. the captured mosquitoes kept alive and transferred on ice to laboratory.

The number of collected mosquitoes per time reach to 200 per time. The morphological characters of captured mosquitoes were identified according to published keys [18]. The most captured mosquitoes were Aedes and Culex spp. Subsequently, Culex spp. were pooled with maximum number of 100 individual per pool according to collection site. The captured mosquitoes were distributed in 5 pools and preserved at −20 °C. Each pool was suspended with 1 mL PBS in Eppendorf tube contain steal balls. The samples were well mixed for 30 s using vortex, then transferred 200 µL aliquots from each mixture for extraction of RNA of WNV.

2.4. Serological assay

Commercial ELISA kit (ID Screen West Nile Competition Multispecies; IDvet Innovative Diagnostics, Grables, France) was used to investigate WNV IgG level in collected serum samples according to the manufacturer's instructions.

2.5. Extraction of WNV RNA

The RNA of WNV was extracted from each aliquot using QIAamp viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

2.6. Reverse transcription PCR (RT-PCR) assay

The RT-PCR assay was performed using specific pair of primer targeting nonstructural protein 5 (NS5) of WNV as previously described by [19]. The PCR reaction was performed in 25 µL volume. For single reaction, the master mix contain 5 µL 5Xbuffer, 1 µL from each primer (20 pmol), 1 µL hotstar Taq DNA polymerase and 12.5 µL OneStep RT-PCR (Qiagen, Hilden, Germany). Finally, 4 µL RNA template were added.

Reverse transcription was conducted at 50 °C for 30 min, followed by a denaturation step at 95 °C for 15 min. Afterwards, the amplification of cDNA was carried out in 40 cycles with initial denaturation for 40 s at 94 °C, annealing for 50 s at 57 °C, and DNA extension at 72 °C for 1 min. The amplified PCR product was examined using 1.5 % agarose gel.

2.7. Sequence and phylogenetic analysis

The purified PCR product was sequenced using the ABI PRISM® BigDyeTM Terminators v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The nucleotide sequences were edited using BioEdit program, then identified against database in gene bank using BLAST. The phylogenetic analysis for the obtained sequence was

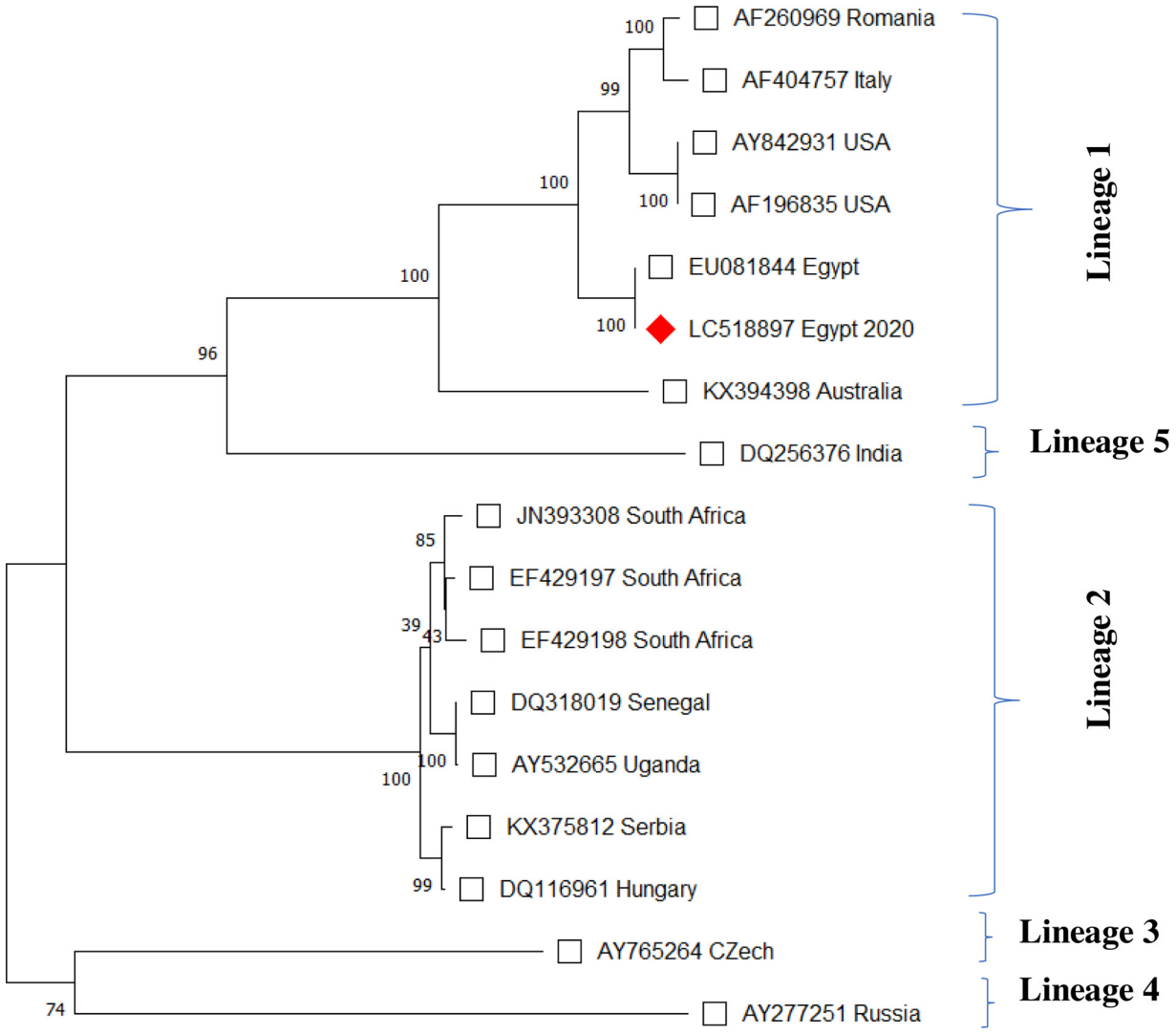

Fig. 2. Neighbor-joining analysis of partial West Nile virus sequence, GenBank accession numbers and geographic origins of strains are shown.

performed using MEGA7 software based on the neighbor-joining tree method with 1000 bootstrap replicates.

2.8. Statistical analysis

The serological results were analyzed using SPSS V17 based on Chi-square test. The results were considered statistically significant at $P < 0.05$.

3. Results

3.1. Seroprevalence of WNV in different localities

The seroprevalence of WNV in different localities showed presence of antibodies against WNV with non-significant difference ($P = 0.1$). Moreover, the highest infection rate was in Qalyubia governorate (25.5 %, 95 %CI: 17.21 – 36.03) followed by Kafr Elsheikh (21.25 %, 95 %CI: 15.35 – 28.56) but it was (17.5 %, 95 %CI: 14.4 – 25.74) and (14.6 %, 95 %CI: 9.25 – 22.14) in Menofia and Gharbia governorates as in Table 1.

3.2. Molecular detection of WNV in mosquito

The captured mosquitoes were divided in five pools and examined with RT-PCR targeting NS5. One pool which collected from Kafr Elsheikh gave positive band with RT-PCR.

The sequence of the obtained WNV isolate has been submitted to GenBank under accession number LC518897. Alignment analysis of the obtained sequence (LC518897) revealed that high homogeneity with WNV EG101 from Egypt and West Nile virus strain PTRoxo from Portugal with similarity percent over 99 %.

The phylogenetic analysis revealed that the analyzed WNV strain belongs to WNV lineage 1. Moreover, it showed close relation between Egyptian strain and Romanian and Italian WNV strains as shown in Fig. 2.

4. Discussion

West Nile Fever caused by WNV which transmitted mainly by Culex spp. The virus can be maintained in nature in a mosquito-bird-mosquito transmission cycle [20]. WNV has been reported in many countries worldwide including Europe, Asia, Africa and middle east [1,12,14]

In Egypt, some studies have been reported the serological prevalence of WNV among horses and donkey s in some localities in the north of Egypt [3].

Subsequently, the present study aimed to wide investigate the seroprevalence of WNV among horse in the north of Egypt and molecular characterization of WNV in mosquitoes.

The present findings confirmed presence of antibodies against WNV among horse in some localities without significant difference at north of Egypt. The prevalence rate was highest in Qalyubia governorate in comparison with other (Kafr Elsheikh, Menofia and Gharbia) governorates. The obtained result was in accordance with Selim et al. [3], they determined the prevalence rate (28.3 %, 22.5 %, 18.5 % and 17.5 %) among horse in Qalyubia, Kafr Elsheikh, Menofia and Gharbia governorate in Egypt. A similar pattern of results obtained by Lafri et al. [21], they detected WNV-antibodies 26.8 % in Algerian horse.

In contrary, our findings lower than previous reported rate (82.6 %) by Azmi et al. [13] among horse in neighbor countries as Israel and Palestine.

The study was carried out in north of Egypt that have different climatic and geographic features. Furthermore, the study area is agricultural and wetlands area, which provide suitable habitats for surviving and multiplication of mosquitoes. Subsequently, this area considers highly potential area for WNV infection [22–27].

The obtained sequence of Egyptian strains (LC518897) was compared with WNV strains in database of GenBank. The phylogenetic tree identified clustering of isolates in 5 lineages. In addition, results explored that Egyptian WNV strain belonged to lineage 1 and all sequences of lineage 1 are geographically distinct [10]. Those WNV strains of lineage 1 were globally spread and isolated from Europe, Africa and America. However, the lineage 2 strains have been recently spread in Austria, Hungary and Greece causing some outbreak among human especially in Greece [28]. Besides lineage 1 and 2, there are lineage 3 which reported in the Czech Republic [6], lineage 4 has been isolated from Russia [7] and lineage 5 was isolated and identified from India [8].

Overall, the sequence analysis revealed similarity between LC518897 and EU081844 Eg101 which was previous isolated from Egypt Egypt [16].

In addition, there is limited diversity in current sequences compared to previous reported data. The present results suggest that circulating WNV strain are genetically conserved with a limited sequence diversity between strains

5. Conclusion

The findings of present study confirm the presence of WNV among horse and mosquitoes. The findings may be due to high density of different mosquito species especially Culicidae family and high population of horse at north of Egypt. Subsequently, it is necessary to implement control program to prevent spreading of WNV and improve public health awareness.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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