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RESEARCH ARTICLE

The Recent Update of the Situation of West Nile Fever among Equids in Egypt after Three Decades of Missing Information

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A B S T R A C T

West Nile virus (WNV) is a viral disease transmitted by mosquitoes to equids and has a zoonotic impact on humans. Although the WNV infection was reported in many countries in the Middle East; a little is known about its prevalence in equine populations in Egypt for the last three decades. We have carried out serosurvey on 400 horses and 150 donkeys in five governorates located in northern Egypt. Antibodies against WNV were found in 83 samples (seroprevalence 20.7%) in horses and 19 (seroprevalence 12.7%) in donkeys. Some risk factors for seropositivity to WNV infection in Egypt as the breed, age, and sex of horses were identified; it is more prevalent among gelding, mixed breed, and the middle age of horses. The infection could be attributed to the absence of control measures or vaccine programs besides the suitable habitat which enhances vector bioavailability. This study revealed the circulation of WNV in northern Egypt; as a result, there is a potential risk of exposure to human populations and there is a necessity for further assessment of the disease circumstances in the upcoming years in Egypt to control it.

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INTRODUCTION

West Nile Virus (WNV), Flavivirus: Flaviviridae, is an important mosquito-borne virus inducing a zoonotic disease called West Nile fever (WNF). The virus is transmitted by mosquitoes as biological vectors, birds as a natural reservoir, and equines and humans representing dead-end hosts (Calistri *et al*., 2010; Benjelloun *et al*., 2017). The temperature plays an important role in speeding the cycle, the transmission period, as well as on the survival of mosquitoes (Elhaig *et al*., 2017; Selim *et al*., 2018); whenever the local ecological conditions are appropriate for viral amplification, humans and equines could easily be infected (Komar *et al*., 2001; DeGroote *et al*., 2014). Mosquitoes, especially *Aedes, Ochlerotatus*, and *Culex* species, transmit WNV, which rapidly spread throughout the US following its introduction in the late 1990s (CDC 2018).

WNF manifested as asymptomatic or mild febrile illness in humans (Ludolfs *et al*., 2007). WNF is a serious illness for the elderly with several reports of deaths (CDC 2018). Also, WNV is a cause of animal disease, especially in equids (horse and donkey) (Azmi *et al*., 2017). The most of infected horses with WNV remain asymptomatic while clinical signs could develop in 10% of infected horses varying from mild fever to severe neurological signs (Pourmahdi *et al*., 2013) as follows: ataxia, paresis in for and/or hind limbs, recumbency and death as a result of the lesions in central nervous system. Many horses show weakness, cranial nerve deficits, and fasciculation of the muscles. Fever is not an ongoing disease feature in horses (Durand *et al*., 2016; Lafri *et al*., 2017). The virus became endemic in recent decades in Europe, Africa, Asia, the Middle East, and Australia.

After its introduction in the US in 1999, WNV induced a severe meningoencephalitis epidemic hitting New York City, then the disease spread to Canada and Central America (LaDeau *et al*., 2007). The Middle East is vulnerable to the spread of endemic and zoonotic diseases because it is located at the core of three contents; WN disease and virus were recorded in such area including Egypt, Iraq, Lebanon, Israel, and the United Arab Emirates (Corwin *et al*., 1992; Alfaresi and Elkoush, 2008; Calistri *et al*., 2010; Ahmadnejad *et al*., 2011; Azmi *et al*., 2017; Hemida *et al*., 2019).

The diagnosis of acute WNV infections is based on the detection of the viral genome by RT-PCR or IgM antibodies by ELISA. Nonetheless, the viral RNA is not easily detected by RT-PCR due to the low virus levels in viremic animals and the short period of viremia in infected horses (Selim *et al*., 2014; Durand *et al*., 2016). Consequently, WNV diagnoses in horses are usually achieved by serological tests, which able to detect IgM antibodies (Abutarbush and Al-Majali, 2014).

WNV was first isolated from humans and mosquitoes in North Cairo, Egypt in 1950 followed by several large outbreaks in Egypt from the period of 1951 to 1954 (Taylor *et al*., 1956). In 1989, a Nile-Delta-seroprevalence study reported only 3% of schoolchildren, 8-14 years of age, had been infected with WNV (Corwin *et al*., 1992). According to these pieces of evidence, Egypt has been listed as a WNV infected country, but there has been some missing information about the situation of the virus in Egypt over the last thirty years and its presumed harmful effects. To fulfill such a gap, this study aimed to determine the seroprevalence of WNV infection in horses and donkeys in different localities in northern Egypt and its related risk factors.

MATERIALS AND METHODS

Blood samples were collected after the consent of the owners and approval of the Faculty of Veterinary Medicine Internal Ethics Review Committee, Benha University, Egypt.

Sample collection and preparation: The sample size was 139 animals with an estimated prevalence of 10% and 5% of accepted errors, based on Win episcope 2.0. For getting more accurate results, we increased the sample size to be 400 horse and 150 donkeys with no vaccination history as equids have not been vaccinated against WNV in Egypt. Animals were randomly selected from five governorates in North Egypt (Giza, Menoufia, Qalyubia, Kafr El Sheikh, and Gharbia) during 2018, shown in the map in Fig. 1.

The blood samples were collected in sterile vacuum tubes without anticoagulant from each animal's jugular vein. Sera will be separate from clotted blood samples by centrifugation (3000 xg for 10 min) and stored at -20^{o}C until use. Because horses are valuable animals, the risk factors related to WNV were investigated. Data were collected from farm managers for each horse, including gender, race, age, and location. The study was applied to Arabian, thoroughbred, and mixed horses, as well as donkeys, breed in Egypt.

Serological tests: The collected serum samples were tested to determine WNV IgG level via a commercial ELISA kit (ID Screen West Nile Competition Multi-species; IDvet Innovative Diagnostics, Grables, France), according to the manufacturer's instructions.

Statistical analysis: The different variables were assessed using multivariable logistic regression and univariable was performed by chi-square analysis; at $P<0.05$, the differences were considered statistically significant.

RESULTS

Seroprevalence of WNV in different localities: The collected serum samples were examined for IgG WNV antibodies during 2018-2019. The seroprevalence of WNV in horse and donkey varied considerably between governorates under the study. Regarding the highest seroprevalence of WNV in horses was found in Qalyubia with 17 out of 60 positive samples (28.3%). While in Giza 37 out of 190 horse sera samples were positive (19.5%), in Menoufia 13 out of 70 horse sera samples were positive (18.5%) and in Kafr El-Sheik 9 out of 40 horse sera samples were positive (22.5%). Also, the lowest seroprevalence was reported in Gharbia 17.5%, Table 1.

Moreover, the seroprevalence of WNV antibodies in donkeys revealed the presence of the virus in the examined areas. The highest seroprevalence was found in the Qalyubia governorate (16.7%) while the lowest seroprevalence area was found in Giza and Gharbia governorates (10%), Table 1.

Table 1: Prevalence of WNV antibodies in horses and donkeys in different localities in Egypt, 2018

Location	Number of horses	No of positive (%)	P-value	Number of donkeys	No of positive (%)	P-value
Giza	190	37 (19.5%)		20	2 (10%)	
Menoufia	70	13 (18.5%)		35	4 (11.4%)	
Qalyubia	60	17 (28.3%)		30	5 (16.7%)	
Kafr El Sheikh	40	9 (22.5%)		45	6 (13.3%)	
Gharbia	40	7 (17.5%)		20	2 (10%)	
Total	400	83 (20.7%)	0.4	150	19 (12.7%)	0.9*

*The results are not significant at P>0.05.

Table 2: logistic regression analysis for the association between each variable and the seroprevalence status of WNV infection in horses

Variable	Category	No	No of positive (%)	Odds ratio	95%CI	P-value
Breed	Arabian	60	8 (13.3%)	ref[a]	-	
	Thoroughbred	150	28 (18.7%)	1.49	0.64: 3.49	0.3*
	Mixed	190	47 (24.7%)	2.14	0.95: 4.82	0.06**
Age	<5 years	130	21 (16.2%)	ref[a]		0.01**
	5-10 years	220	54 (24.5%)	2.17	1.24: 3.78	0.006**
	>10 years	50	8 (16%)	1.37	0.56: 3.34	0.4*
Sex	Male	190	51 (26.8%)	ref[a]	-	
	Female	150	13 (8.6%)	0.259	0.135: 0.497	0.0001**
	gelding	60	19 (31.6%)	1.26	0.67: 2.37	0.4*

[a]ref = reference category; *The results are not significant at P>0.05. *The results are significant at P<0.05.

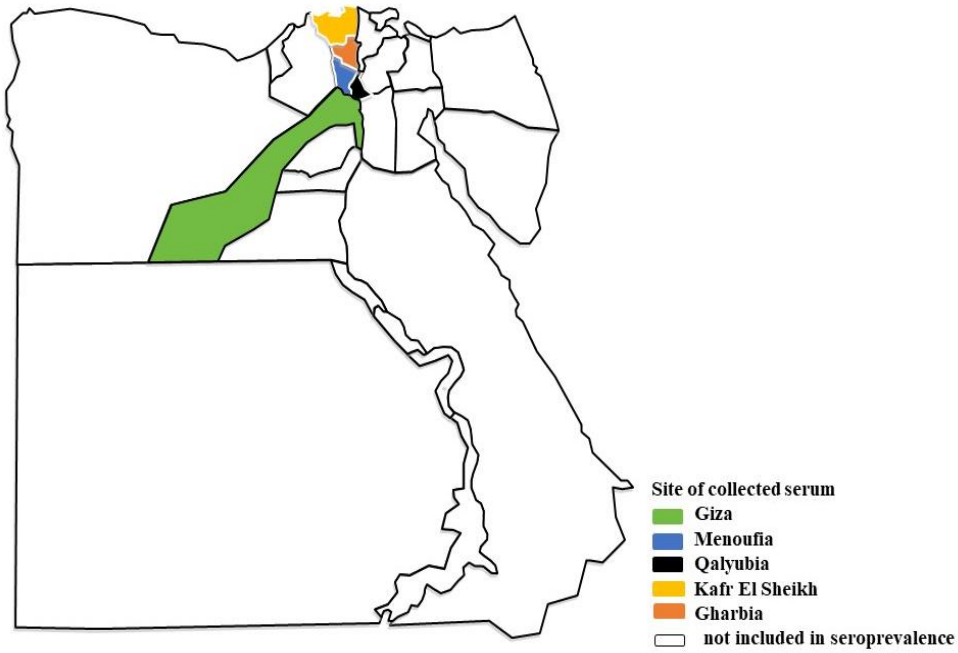

Fig. 1: Surveyed locations for WNV in northern Egypt.

Risk factors related to WNV among horses: In the unconditional logistic regression analysis, the results varied according to the variable studied (Table 2). For the breed variable, the mixed breed (OR=2.14, P=0.06) showed the highest effect on the seroprevalence of WNV infection among horses. Moreover, animals in the middle age group, 5-10 years, (OR=2.17 P=0.006) were significantly associated with the prevalence of the WNV infection. The risk factor was decreased in older animals, >10 years age, (OR=1.37 P=0.4). Regarding sex, the gelding was found to be associated with a high risk of infection for WNV (OR=1.26 P=0.4) but did not differ in females.

DISCUSSION

Parasites, especially mosquitoes (Diptera: Culicidae), since ancient times, are related to serious threats to humans and animals because they are vectors for chronic, debilitating, and even disabling diseases leading to overwhelming financial losses worldwide (Khater, 2017).

Mosquitoes do transmit parasites for instance malaria and filariasis and important arboviruses as WNV. Mosquito-borne diseases by and large expand to new areas all over the globe because people plus goods are moving around at increasing speed and rates; the development of resistance in mosquitoes and the fast spread of pathogens lead to overwhelming epidemics and enormous challenges (Benelli *et al*. 2015). Therefore, mosquito safe control through larvicides (Khater and Shalaby 2008; Selim *et al*., 2013; Roni *et al*., 2015) and repellents (Khater *et al*., 2019) is an urgent need.

The first report of WNV in Egypt was among humans in 1950 followed by several reports of the disease during 1951, 1954, 1968 and 1989 (Southam and Moore 1954; Taylor *et al*. 1956; Corwin *et al*., 1992); whereas the disease has been reported in horses of some neighboring countries such as Palestine (Azmi *et al*., 2017), Jordan (Abutarbush and Al-Majali, 2014), and Saudi Arabia (Hemida *et al*., 2019).

This is the first study after missing data for three decades to investigate the prevalence of WNV and evaluate the risk factors thought to be attributed to the occurrence of such disease among the Egyptian horses. The seroprevalence was performed in five governorates Northern Egypt in both horses and donkeys. Anti-WNV antibodies were detected in sera of 83 horses out of 400 tested with a 20.7% seroprevalence rate while were detected in sera of 19 donkeys out of 150 with 12.7% seroprevalence rate.

The seroprevalence of WNV infection in horses and donkeys was non-significantly different in localities under study despite the higher prevalence in Qalyubia and Kafr Elsheikh governorates, probably due to the availability of the *Culex* spp. in such areas, but *Aedes* spp. is not prevalent in Egypt (Abozeid *et al*. 2018).

Overall our findings are lower than previous findings reported by Schmidt and Mansoury (1963) for seroprevalence of WNV among horse population in Egypt, They found high titer of anti-WNV antibodies in three species of equids, 67% in horses, 47% in donkeys and 44% in mules. Also, Hemida *et al*. (2019) found high antibodies titer against WNV in some localities in Saudi Arabia. Furthermore, our findings are similar to the previously reported prevalence rates, 26.8 and 14.4%, among Algerian horses and donkeys, respectively (Lafri *et al*. 2017).

Besides, our findings were higher than the seroprevalence rates reported in a newly infected area, such as New York City in 1999 (2.7%) (Komar *et al*., 2001) and lower than other rates reported in horse (82.6%) and donkeys (39.3%) in neighbor countries as Israel and Palestine (Azmi *et al*., 2017).

In the present study, the disease is serologically detected in different localities in northern Egypt may be due to high humidity and temperatures which provides a suitable habitat for the presence of potential WNV competent vectors, such as *Culex* spp. and the other mosquito-vector species.

The seroprevalence also varied within horse individual characteristics. The thoroughbred and mixed breeds showed a higher risk for WNV infection in this study. Ahmadnejad *et al*. (2011) recorded similar results attributed to the presence of a high population density of mosquitoes among such breeds of horses. Also, we speculate that this might be due to various management conditions of each animal.

Concerning the age of infected animals, the seroprevalence rate of WNV infection in this study was higher among younger animals (5-10 years old); therefore, middle-age had a risk factor effect on the prevalence of the disease. The gelding is more susceptible to the WNV; whereas females were not associated with the increased risk of WNV infection. Our findings have come along with findings reported by Ahmadnejad *et al*. (2011) but contradict that reported by Abutarbush and Al-Majali (2014) as they did not identify sex, breed, and age as risk factors for seropositivity to WNV infection.

Conclusions: The present serological survey documented the presence of West Nile Fever among equids in northern Egyptian Governorates, mainly in Qalyubia and Kafr El Shiek, after three decades of missing information. It is more prevalent among gelding, mixed breed, and the middle age of horses. Consequently, there is a potential risk of exposure to human populations. Future studies are recommended for better vector control strategies (Govindarajan *et al*. 2016a,b) and crucial monitoring of WNV in Egypt through assessing the situation of the disease in the upcoming few years among humans, equids, birds, and mosquitoes as well as treating infected individuals.

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Authors contribution: AS, AR and FA helped in designing, sample collection, laboratory work and writing the work; HK helped in editing and publishing the work.

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