



# The first detection of anti-West Nile virus antibody in domestic ruminants in Egypt

Abdelfattah Selim<sup>1</sup> · Abdelhamed Abdelhady<sup>2</sup>

Received: 16 January 2020 / Accepted: 17 June 2020 / Published online: 25 June 2020  
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## Abstract

West Nile virus (WNV) is a mosquito-borne disease, usually present as a symptomatic disease but can cause various clinical signs ranged from mild fever to severe encephalitis and death in various animals and humans. In Egypt, the epidemiological data about WNV infection in different animal species particularly in domestic ruminants are scarce. The present study aimed to investigate the seroprevalence of WNV in cattle, buffalo, camel, sheep, and goats at some Governorates northern Egypt. In total, 360 serum samples (100 cattle, 50 buffalo, 50 camels, 85 sheep, and 75 goats) were examined using ELISA. The results revealed that the seroprevalence of WNV among ruminants was highly significant ( $P = 0.03$ ) at Kafr El Sheikh Governorate (17.6%) in comparison with other the Governorates. Besides, the seroprevalence of WNV antibodies significantly differed between the examined species ( $P = 0.0001$ ); it was 22%, 0%, 40%, 3.5%, and 5.3% in cattle, buffalo, camel, sheep, and goats, respectively. This is the first study to confirm that domestic ruminants act as a reservoir in the epidemiology of WNV infection and represent a risk for human and equine infections in Egypt.

**Keywords** West Nile virus · Serology · Ruminants · Egypt

## Introduction

West Nile virus (WNV) is a mosquito-borne zoonotic pathogen and belongs to the Japanese encephalitis virus serocomplex within genus *Flavivirus*, family *Flaviviridae* (Davoust et al. 2016; Selim et al. 2020b). WNV is mainly transmitted by *Culex* spp. of mosquitoes which act as a bridge between humans and birds (Baba et al. 2014). Mosquitoes act as vectors, while birds act as reservoirs within the transmission cycle of the disease, whereas the virus could be replicate in birds and mosquitoes which considered a source of infection to animals and humans (Oluwayelu et al. 2018; Sule et al. 2018).

Human and equids are accidental and dead-end hosts. The infection is usually asymptomatic or mild in humans, but it

could be ranged from slight incoordination and muscle weakness to severe ataxia and recumbency in the case of the affected horses (Albayrak and Ozan 2013; Erol et al. 2016).

The disease has frequently appeared in humans and horses. Since then, it has been associated with sporadic and major outbreaks in humans and horses (Erol et al. 2016; Selim et al. 2020a).

The experimental infections by WNV have been shown that the domestic animals rarely developed antibodies titers or clinical signs. During the last three decades, some studies have been detected antibodies against WNV in some of the domestic animals like cattle, camels, and goats in natural conditions (Jupp 2001; Sule et al. 2018).

Serological diagnostic tests are favored to confirm presence of WNV infection. Many serological tests are available for screening WNV antibodies in domestic animals such as virus neutralization tests (VNT), immunofluorescence assays (IFA), and enzyme-linked immunosorbent assays (ELISA) (Beck et al. 2013). Rapid tests such as IFA and ELISA are preferred because of their sensitivity and reproducibility, but the VNT are still considered the gold standard test and provide highly diagnostic specificity (Beck et al. 2017).

The West Nile fever is distributed worldwide in Southern Europe, Asia, Australia, North America, Africa, and the

✉ Abdelfattah Selim  
Abdelfattah.selim@fvtm.bu.edu.eg

<sup>1</sup> Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Benha University, Toukh, Egypt

<sup>2</sup> Department of Parasitology and Animal Diseases, National Research Center, Giza, Egypt

Middle East (Cardinale et al. 2017). The WNV infection in Egypt was first identified in 1950 in north Cairo, followed by several outbreaks recognized from 1952 to 1954, leading to an understanding of WNV epidemiology in humans (Sayed-Ahmed 2016).

The activity and population of the vectors are high in north Egypt. However, the information about WNV among ruminants in Egypt is absent hence the need for this study which aimed to determine the seroprevalence of WNV in ruminants in some localities north Egypt.

## Materials and methods

### Study area

The present study has been performed in four Governorates located at the Delta area, between the two branches of Nile river, north Egypt as Kafr El Sheikh, Qalyubia, Menofia, and Gharbia located at 38°18 N to 30°56 E; 30°25 N to 31°13 E; 30.52°N 30.99°E; and 30.867°N 31.028°E, respectively (Fig. 1). Such areas have been selected based on their demography allowing the high density of ruminants as well as vector populations, thus spreading the virus between animals. Small farmers in rural areas do mix breeding of at least one species of ruminants like cattle, buffalo, sheep, goat, or camel with a donkey or a horse for transport; such animals are reared in the same place.

### Sample collection

The Win Episcopy 2.0 ([www.winepi.net](http://www.winepi.net)) was used for calculation of sample size to determine the population's prevalence which depends on an expected prevalence of 20% as reported by Selim et al. (2020a) with confidence limits of 95% and expected error of 5%. The calculated samples were 236, and we increased the number to 360 to represent the different species and localities.

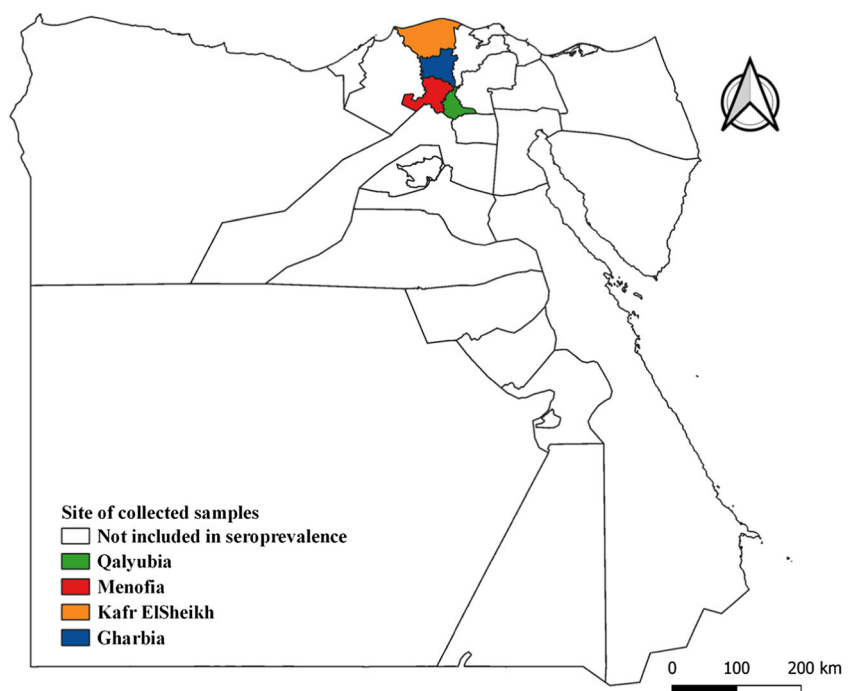
A total of 360 serum samples were collected randomly as follows: 100, 50, 50, 85, and 75 from cattle, buffaloes, camels, sheep, and goats, respectively.

The collected samples represented the four Governorates under the study during the period from July 2018 to June 2019. The blood samples were collected from the jugular vein of each animal into a sterile vacuum tube without an anticoagulant agent. Sera will be separate from clotted blood samples by centrifugation at 1500 rpm for 10 min and stored at  $-20^{\circ}\text{C}$  until use.

### Serological analysis

The serum samples were examined to detect the antibodies against WNV in different animal species using a commercial ELISA kit (ID Screen West Nile Competition Multi-species; IDvet Innovative Diagnostics), according to the manufacturer's instructions.

**Fig. 1** Prevalence of WNV in different surveyed locations in Egypt



The used ELISA kit exhibits high specificity (80–96%) and high sensitivity (100%) as evaluated by Sotelo et al. (2011). In brief, samples and controls were added into wells of coated ELISA plates with WNV antigen. Peroxidase-conjugated anti-WNV antibody was added into each well after incubation and washing of microplates. After incubation, the washing step was repeated to remove the excess of conjugate, and the substrate solution TMB was added into the wells. The optical density was measured by microplate ELISA reader at 450 nm.

$$S/N\% = \text{OD of each sample} / \text{OD of negative control} \times 100$$

A sample having an S/N% less than or equal to 40% was considered positive and greater than 50% considered negative, while S/N% greater than 40% to less than 50% was considered doubtful and should be repeated.

### Statistical analysis

The statistical analysis was performed using SPSS (Ver16, USA) using chi-square test to compare the prevalence of antibodies against WNV in different species. The difference was considered significant at a probability level  $\leq 0.05$ .

### Results

Seropositive animals were detected in the four surveyed Governorates in north Egypt. The serological results of the examined species in the different localities revealed that the prevalence of WNV was significantly higher in Kafr El Sheikh (17.6%, 95% CI 11.59–25.66) when compared with those of the other Governorates, 11%, 10%, and 6.9% in Qalyubia, Menofia, and Gharbia, respectively, Table 1.

The seroprevalence of WNV among ruminants (cattle, buffalo, camel, sheep, and goats) was significantly different, in Table 2. The antibodies against WNV were not detected in buffalo under the study. Moreover, camels had a significantly higher prevalence rate (40%, 95% CI 26.73–54.8) than those of cattle (22%, 95% CI 11.3–27.22), sheep (3.5%, 95% CI 0.92–10.69), and goats (5.3%, 95% CI 1.72–13.8).

### Discussion

WNV is an important mosquito-borne *Flavivirus*, widely distributed in many parts of the world. The WNV infection has been reported in humans and birds in Egypt (Sayed-Ahmed 2016). To date, the epidemiological situation of the WNV infection has not been studied in domestic ruminants. Consequently, the current work investigated the serological prevalence of antibodies against WNV in cattle, buffalo, camel, sheep, and goats in four Governorates located in Delta, Egypt, based on ELISA test.

Most of the positive cases of this study (22 out of 125) were reported in Kafr El Sheikh Governorate in comparison with the other localities. The highest prevalence of WNV infection in Kafr El Sheikh Governorate may be attributed to its unique geographical location as it is located at the Mediterranean Sea acting as a route of entry to migratory birds which play an important role in the transmission of the disease (Erol et al. 2016). Moreover, its demography makes it characterized by high humidity as it is in the delta region and famous with growing rice which is a suitable breeding site for mosquito vectors of WNV which support the present findings (Ozdenerol et al. 2013; Zayed et al. 2015).

The distribution of WNV infection in the localities under the study is comparable with that of Corwin et al. (1992) who detected antibodies against WNV in children, different hosts, and isolate the virus from a mosquito in a study conducted in Nile Delta during 1989.

Moreover, the serological examination of sera of domestic ruminants revealed that detectable antibodies titer in all examined animals except buffaloes, whereas the highest seroprevalence was detected in camels (40%) followed by cattle (22%), sheep (3.5%), and goats (5.3%). Our findings could be explained as buffalo is originally a wild and resistant host, and the other domestic ruminants raised in North Egypt are infected by WNV and act as reservoirs of infections.

The findings of the present study come in accordance with a previous study detected antibodies against WNV in cattle, camel, sheep, and goats in Nigeria (Olaleye et al. (1990). In contrast, some studies did not detect antibodies against WNV in cattle, buffalo, sheep, and goats (Albayrak and Ozan 2013).

**Table 1** Seroprevalence of WNV in different localities northern Egypt

Location	No. of tested animals	Positive	% of prevalence	95% CI	P value
Kafr El-Sheikh	125	22	17.6	11.59–25.66	0.03*
Qalyubia	127	14	11	6.36–18.11	
Menofia	50	5	10	3.74–22.59	
Gharbia	58	4	6.9	2.23–17.55	
Total	360	45	11.7	8.63–15.55	

\* The results are significant at  $p < 0.05$

**Table 2** Seropositivity rate of WNV among domestic ruminants

Location	No. of tested animals	Positive	% of prevalence	95% CI	<i>P</i> value
Cattle	100	18	22	11.3–27.22	0.0001*
Buffalo	50	0	0	0–8.89	
Camel	50	20	40	26.73–54.8	
Sheep	85	3	3.5	0.92–10.69	
Goats	75	4	5.3	1.72–13.8	
Total	360	45	12.5	5.35–16.47	

\* The results are significant at  $p < 0.05$

In addition, the results are directly in line with other studies which detected antibodies against WNV in cattle and buffaloes and sheep (Darwish et al. 1983; Mariéa et al. 2016). However, when comparing our results to those of older studies, it must be pointed out some study could not be detected antibodies in goats, Pakistan (Darwish et al. 1983) but were detected in Tunisian camel (Hassine et al. 2017).

The seroprevalence results approved that the domestic ruminants implicate as useful sentinels for WNV, although the biological basis for this remains unknown. These findings may be attributed to the domestic ruminants present in large herds which attract a greater number of mosquitoes (Ulloa et al. 2009). The domestic ruminants usually are transported for a commercial purpose that gives a chance to develop antibodies against some zoonotic infection (like WNF) from remotely acquired infection (Deegan et al. 2005).

It is interesting to note that WNV antibodies were detected in most of the domestic ruminants in north Egypt playing a role in the maintenance and circulation of the virus among animals (Giadinis et al. 2015).

## Conclusion

Our results act as the first alarm to the veterinarian that ruminants act as a reservoir of infection with WNV and disease is likely to be endemic in Egypt. WNV should be investigated as a potential etiological agent for any fever and neurological outbreaks that may occur among animals and humans. Besides, more efforts should be taken to isolate WNV from its hosts to determine whether or not circulating WNV strains have been attenuated or retain its virulence in Egypt.

**Funding information** The authors wish to thank the Science and Technology Development Fund (STDF) for their financial support through the project Number (34763) to complete this work.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflicts of interest.

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