Occurrence of Equine West Nile Virus Among Horses in Qatar : A Preliminary Investigation

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Abstract

West Nile Virus (WNV) is an emerging threat to public health authorities around the globe. WNV is maintained in ecosystems primarily in enzootic cycles involving mosquito vector and avian hosts, with epizootic spread to mammals including horses and humans. Outbreaks of WNV disease in mammals have been associated with significant losses. The factors that play roles in the evaluation of these outbreaks are not fully known and the disease has not been reported in Qatar. We carried out a study to determine the prevalence of exposure to WNV in the State of Qatar and identify the factors that are likely to associate with seroconverion.

Keywords: Equine West Nile virus, Disease prevalence, Risk factors

Introduction

West Nile fever (WNF) is a viral disease that infects birds, humans

and horses causing an apparent infection, mild fever, encephalitis, meningitis, or death (WHO, 2013). The causal agent, West Nile virus (WNV), is a mosquito born RNA virus classified under the genus *Flavivirus* belonging to the Japanese encephalitis antigenic complex of the family *Flaviviridae* (ICTV, 2014). Originally identified and named by Smithburn and colleagues in 1937, the disease was known in West Nile province of Northern Uganda (IDA, 2004). Before 1999, WNF was only restricted to Africa, Middle East, Asia and East Europe. The first cases of severe human encephalitis caused by WNV were detected in elderly people in Israel in 1957 (Lindsey, Lehman, Staples & Marc Fischer, 2014). Since then, it was known that WNV could cause serious central nervous system infections in humans. In 1999 human cases of WNF including 41% neuroinvasive disease were reported to the Centers for Disease Control and Prevention (CDC) from 47 states and the District of Colombia in the United States (WNF, 2003). Birds are primary amplifier hosts for WNFV (McCoy, 2003) and the disease is maintained horizontally in nature through mosquitoes-birds-mosquito cycling transmission and vertically (CDC, 2010). Being responsible for transmitting infection to horses and humans, which are incidental hosts, 249-mosquito pool out of 4136 were tested positive for WNFV isolation in New York state, USA (Bajwa, O'Connor, Slavinski & Butts, 2014). Some studies indicated that ticks are clearly not efficient vectors of WNFV and therefore are unlikely to be important vectors in U.S (Lawrie, Uzcategui, Gould, & Nuttal, 2004). Contrary to this, a study documented the nonviremic transmission (NVT) of WNFV between infected and non-infected *Ornithodorus moubata* ticks (Higgs, Schneider, Vanlandingham, Klingler, and Gould, 2005). Migratory passerine birds were nominated potential dispersal vehicles for WNV (Owen, Moore, Panella, Edwards, Bru, Hughes & Comar, 2006). However, other birds including rock doves, gulls, robins, mallard ducks, hawks and eagles were tested positive for WNFV in the USA (MMM, 2002).

The fact that Qatar is a peninsula serving as a bridge and land to several migratory avian species, and that there is an increasing activity of importing wild birds in the country should draw attention to WNF epidemiology in the state. With a total number of 1081 approximating 20% of the equine population in the country (DAR, 2016), the active participation of horses in regional and international equestrian festivals of WNF endemic areas should be reconsidered in the light of their potential carriers of the disease always endangering the public health status in Qatar.

The purpose of this preliminary study was to determine the occurrence of WNV in Qatar, investigate possible prevalence of the disease among the Qatari horses, and to elucidate the factors that could play roles in exposure to WNV and their association with seroconversion.

Materials and Methods

Samples and data collection and processing

An approximate of 5-8 mL of blood serum samples were collected by venipuncture of the jugular vein from 260 horses stabled at 6 locations in Qatar in 2006 and 2014. Appropriate available records of each animal including location, age, breed and sex were recorded. Post blood clotting, all samples were immediately chilled and transported for further processing. Sera were prepared following the standard procedure after overnight incubation at 18-26^{o}C. Each sample was aliquot into 2 mL cryovial tubes and stored at -80^{o}C till used.

Determination of WNV antibody

The enzyme-linked immunosorbent antibody (ELISA-Ab) assay was used for determination of WNV IgM and pE Ab in the collected blood sera using WNIGM VER 0111 GB ELISA-Ab and WNC VER 0110 ELIA-Ab kits, respectively (IDVET, Innovative Diagnostics France). Both procedures followed the manufacturer's instructions. Determination of the results Using a 450nm WL-aided BioTek ELx808 spectrophotometric microplate ELISA reader, results obtained as optical densities were recorded, and the results were interpreted using Gen5.5 software version and a validated spreadsheet ELISA interpretation calculator.

Statistical Analysis

The prevalence of seroconversion to exposure to WNV was computed as the proportion of horses that tested positive out of all the samples that were examined. The significance of association between each factor and the likelihood of seroconversion to WNV was assessed us the univariate logistic regression. Factors that were significant in the univariate analysis were evaluated together in a multivariate to assess the effect of each factor while simultaneously controlling for the significance of other factors. The analysis was performed using the multivariate logistic regression analysis. The magnitude of the association between each factor and the likelihood of seroconversion was quantified using the odds ratio (OR). All significances were considered at type I error of $\alpha \leq 0.10$.

Results

All of the 260 horses were negative to WNV-IgM Ab eliminating evidence of recent exposure to WNV. 61/260 of the samples showed evidence of seroconversion to WNV-pE-Ab with a prevalence of 23.5%. The seroconversion rate of each of the samples collected from the 6 locations was shown in Figure 1. Within these locations the prevalence varied between 10% in location 2 to 35% in location 1. 15/60 of the horses

sampled from location 6 indicated evidence of seroconversion. Unfortunately, data on the other risk factors were not complete and these horses were not considered in any further analysis.

The significance of association between each factor and the likelihood of seroconversion to WNV are shown in Table 1. It was twice more likely to detect seroconversion to the WNV among horses samples from location 1 in comparison to horses sampled from location 5. All the other locations had a lower likelihood of seroconversion to WNV in comparison to location 5, but the risk was not significant (Table 1).

There was a significant association between the breed of the horse and the likelihood of seroconversion to WNV (Table 2). It was twice more likely to detect antibodies to WNV in samples collected from Thoroughbred horses compared to samples collected from Mixed breed horses (OR = 2.2).

Discussion

The primary objective of our study was to examine the possible evidences of exposure to the WNV among horses in Qatar and to determine the factors that could be associated with seroconversition to WNV. Although exposure to the virus had been reported before in other parts of the world, no evidence or report on the disease had been cited in Qatar. Noting that there is no report of the disease either in horses or in human in the Arabic Penensila, evidence of exposure to the virus had been reported in neighboring countries including the United Arab Emirates, Iran, and Pakistan (Chinikar et al., 2013; Zohaib et al., 2015; Joseph et al., 2016).

Being the first report of exposure to WNV in Qatar , none of the sampled horses had a history of vaccination against WNV. As well, it was surprising enough to our observation that there was high seroconversion to WNV among the tested samples. Compared to the large difference in seroconversion proportion to WNV between horses sampled in Iran and Pakistan, WNV antibody prevalence among horses in Qatar somewhere fall in the middle. The factors that could account for the differences among these countries were not known. However, one speculative explanation could contribute to the disparity is the differences in the targeted animal species among the populations in these countries. Another factor could be the abundance of types of mosquito vectors in the ecological niches where the samples were collected (Moser et al., 2016).

Given that the investigation applied a competitive EISA technology detecting presence of anti-WNV-pE Ab and an indirect ELISA capture assay showing absence of anti-WNV-IgM Ab in these sera (CDC, 2015), the logica interpretation is the persistence of WNV-IgG Ab in the sera of these horses. The epidemiological data that eliminating previous exposure of these

animals to other encephalitis complex diseases would augment the rationale of specificity of these Ab to WNV.

We evaluated several risk factors that were expected to predispose to the likelihood of sroconversion to WNV. Among the breed of the horses there was high likelihood of seroconversion among throughbreds. None of the horses included in the study were vaccinated against WNV which leads to the conclusion that the serconversion is real. Although there is no published data on the presence of the mosqueto vector, the personal communication with entomologists in the country indicated the presence of Culex mosqueto. Also, with Qatar being a magnet to a lot of immigrants and several flights arrive daily in the country, with some brining live animals, one would not stamp out the presence of the mosqueto vector. Additional, while no data is available on the seroconversion among birds, the ampiliphying hosts of the virus, Qatar is known to be annual migiratory and exotic birds resort.

Conclusion

This study demonstrated for the first time that horses in Qatar have been exposed to the WNV hinted at the possibility that the virus might be endemic in the country and has a potential to pose risk to human health.

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Ethical declaration

This is to certify that the authors of this submitted manuscript performed the study in accordance with the ethical standards laid down in the 1964 Helsinki Declaration and its later amendments. Moreover, all authors approved their consent prior to conduction of the investigation.

Conflict of interest

The authors declare that there is no conflict of interest at individual or institutional levels concerning the investigation.

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Author contribution

The authors were contributed equally to the manuscript revision. MH and HO were contributed to the design of the investigation, analysis of the ELISA results and the statistical analysis, respectively and manuscript writing; ED contributed to design of the ELISA results validation and interpretation spread sheet; AS was contributed to data collection and ELISA performance.

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Table 1. The association between each of the putative risk factors and the likelihood of seroconversion to WNV in locations 1 to 5.

Risk factor	Number positive	Number negative	Odds ratio (95%Cconfidence interval) I
Location			
One	24	44	2.3 (1.0, 5.2)
Two	2	18	0.5 (0.1, 1.9)
Three	9	41	0.9 (0.4, 2.4)
Four	4	21	0.8 (0.3, 2.5)
Five	7	30	1.0
Breed			
Arabian	22	87	(0.5, 2.2)
Thoroughbred	15	29	2.2 (1.0, 4.9)
Mixed	9	38	1.0
Sex			
Mare	33	80	1.6 (0.9, 3.0)
Gelding	1	27	0.1 (0.03, 0.8)
Stallion	47	47	1.0
Age (years)			
5<	4	34	0.4 (0.1, 0.9)
5-10	21	52	1.3 (0.7, 2.4)
>10	21	68	1.0

Table 2. Factors significantly associated with the likelihood for seroconversion to WNV in the multivariate logistic regression analysis among subject horses in locations 1 to 5.

Risk factors	Regression coefficient (Standard error)	Adjusted odds ratio and 95% confidence interval
Sex		
Mare	0.598 (0.411)	1.8 (0.9, 3.6)
Gelding	-1.820 (1.086)	0.1 (0.03, 0.9)
Stallion	0.0	1.0
Breed		
Thoroughbred	-0.663 (0.398)	0.5 (0.3, 0.9)
Arabian	0.0	1.0
Age (years)		
5<	-1.079 (0.597)	0.3 (01, 0.9)
5-10	0.247 (0.382)	1.3 (0.7, 2.4)
>10	0.0	1.0

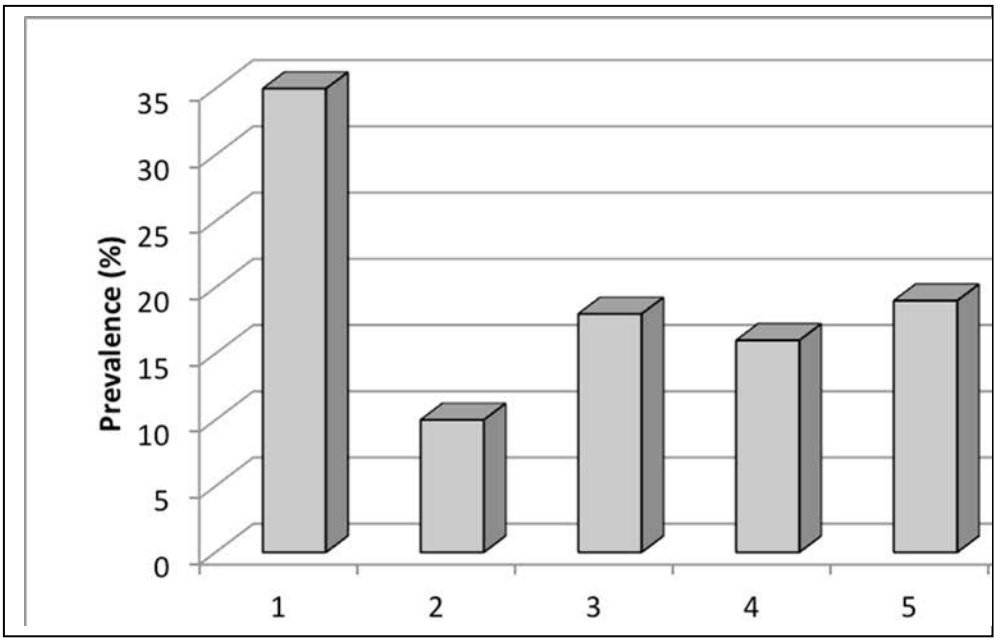

Figure (1): WNV PrE-Ab prevalence rates among the subject horses at the six different locations tested at the survey duration.