High Seroprevalence of West Nile Virus Antibodies Observed in Horses from Southwestern Nigeria

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Abstract

To investigate exposure of Nigerian horses to West Nile virus (WNV), we determined the seroprevalence rate of anti-WNV antibody in a cohort of 145 horses. Serum samples were collected from three locations in southwestern Nigeria between October, 2011, and July, 2012. The horses were asymptomatic and unvaccinated against WNV at the time of sampling. All sera were tested using a competition enzyme-linked immunosorbent assay (ELISA) and by an immunoglobulin M (IgM)-specific ELISA. High rates of anti-WNV antibody prevalence were observed in all locations with a mean level of 90.3% (95% confidence interval 84.3–94.6%). None of the horses had detectable anti-WNV IgM. This is the first ELISA-based report of WNV seroprevalence in Nigerian horses and suggests that WNV is enzootic in the study areas, indicating a potential risk of infection in humans and animals.

Key Words: Horse—Anti-West Nile virus antibody—Seroprevalence—ELISA—Nigeria.

Introduction

WEST NILE VIRUS (WNV) IS A mosquito-borne virus and belongs to the genus *Flavivirus* of the family *Flaviviridae*. The viral genome is enclosed by a capsid, which is surrounded by a host-derived envelope that has been modified by two membrane glycoproteins—the precursor membrane (prM) and envelope glycoprotein (E). The virus is maintained in an enzootic cycle between ornithophilic mosquitoes and birds. Infection in horses and humans occurs if they receive a bite from a WNV-infected female mosquito (Lim et al. 2011, Qian et al. 2014). The E protein mediates virus–host cell interaction/binding; it also induces host immune response and is the most immunologically important structural protein of the virus (Martin-Acebes and Saiz 2012). WNV infection of mammalian hosts induces both innate and adaptive immune responses, with the latter being commonly measured by detection of immunoglobulins (Ig) M and G (Kleiboeker et al. 2004, Dauphin and Zientara 2007). Serological surveys enable monitoring and prompt reporting of WNV activity in a location, and such studies have reported the presence of the virus in all continents of the world. One of the earliest reports of WNV infection occurred

in a 12-year-old police horse in Egypt in 1959 (Murgue et al. 2002). Since then, WNV outbreaks in humans, birds and horses have been reported in Greece, Hungary, Spain, and central Europe (Papa et al. 2010, Kutasi et al. 2011, Lopez et al. 2011, Bakonyi et al. 2013). In sub-Saharan Africa, WNV activity in horses has been documented in Senegal, Côte d'Ivoire, Chad, Democratic Republic of the Congo, Gabon, and Djibouti (Cabre et al. 2006), whereas in South Africa, diagnosis and seroprevalence studies of WNV in horses have been reported (Venter et al. 2011). In Nigeria, Olaleye et al. (1989) detected anti-WNV complement-fixing antibodies in 71% of 62 adult male horses from two stables in Lagos (southwestern Nigeria).

In a recent report of WNV infection among humans in semiarid northeastern Nigeria, Baba et al. (2013) tested blood samples from humans in Maiduguri, Borno State, during the rainy (July to September) and dry harmattan (October to March) seasons and obtained a WNV seroprevalence level of 25%. However, despite the fact that horses are prized animals that are susceptible to WNV-induced neurologic disease, there has been a dearth of information since the 1990s on WNV in southern Nigeria, where there are estimated to be more than 1 million equids (Bourn et al., 1994).

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TABLE 1. ANTI-WEST NILE VIRUS ANTIBODY PREVALENCE RATES AMONG HORSES IN NIGERIA

Location	Percentage seroprevalence (n)						
	Overall	Sex		Breed		Year	
		Male	Female	Criollo	Dongola	2011	2012
Ibadan	97.8 (45)	100 (17)	96.4 (28)	—	97.8 (45)	—	97.8 (45)
Ikoyi	83.5 (79)	62.5 (8)	85.9 (71)	58.3 (24)	94.5 (55)	96 (25)	77.8 (54)
Ajah	100 (21)	100 (4)	100 (17)	100 (20)	100 (1)	100 (21)	—
Total	90.3 (145)	87.5 (29)	94.1 (116)	79.2 (44)	97.4 (101)	98 (46)	87.8 (99)

Data shown are from three locations—Ibadan, Ikoyi, and Ajah. Numbers in parentheses are the number of horses tested.

There is also a lack of information on seasonality of WNV vectors in Nigeria. Therefore, this study was conducted to determine the prevalence rate of anti-WNV antibody in apparently healthy/asymptomatic polo horses in south-western Nigeria.

Materials and Methods

Blood samples were collected from 145 healthy horses selected consecutively between October, 2011, and July, 2012, at three locations in southwestern Nigeria. The locations represent areas where a large number of horses were present and used for polo, and all available horses except those with history of WNV vaccination were sampled. The locations selected were the Ajah horse stable (N06.46700°, E003.57255°) and the Ikoyi polo club (N06.4500°, E003.4333°), both in Lagos State, and the Ibadan polo club (N07.4036°, E003.8726°) in Oyo State. The climate of the study locations is tropical, with Lagos State receiving approximately 1800 mm of rainfall annually and Oyo State an average of 1200 mm at the onset but 1800 mm at the peak of rainy season (Soladoye and Oromakinde 2013, Adeniyi et al. 2014). The period of sample collection coincided with the wet season.

Within the cohort, no history of febrile illness or neurological disease was reported prior to sampling. The horses were all adults (≥ 3 years; most of them older than 8 years) housed under comparable condition, with females comprising 80.0% ($n=116$) of the cohort. Two breeds were sampled—the Argentine Criollo (imported as adults) and the local Dongola breed (born and nurtured in Nigeria). All horses were privately owned and unvaccinated against WNV. Blood samples were collected by jugular venepuncture and separated sera were stored at -20°C prior to testing. The sera were screened for the presence of total anti-WNV antibody using the ID Screen® West Nile Competition Enzyme-Linked Immunosorbent Assay (cELISA; IDVET, France), whereas IgM was detected using the West Nile Virus IgM Antibody Test Kit (IDEXX, Montpellier SAS, France). Positive samples were confirmed by the plaque reduction neutralization test (PRNT), as described previously (Mansfield et al. 2011).

Results obtained were presented with descriptive statistics with 95% exact binomial confidence interval (CI) where applicable. Statistical associations were determined using binary logistic regression analysis to estimate odds ratios (OR) with 95% CI. Associations with a p value ≤ 0.05 were considered significant. The analysis was performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, IL). The study design and methodology were approved by the Animal

Care and Use Research Ethics Committee of the Faculty of Veterinary Medicine, University of Ibadan, Nigeria.

Results and Discussion

This study was conducted to assess WNV seroprevalence in horses more than two decades after the last seroprevalence studies in Nigerian horses (Olaleye et al. 1989, 1990, Omi-labu et al. 1990). Overall, WNV seroprevalence obtained for the three locations tested was 90.3% (95% CI 84.3–94.6%), as anti-WNV antibodies were detected in 131 of the 145 horses tested. Within individual locations, seroprevalence varied from 100% in Ajah to 83.5% (95% CI 73.5–90.9%) in Ikoyi (Tables 1 and 2). Of all the four variables analyzed, only breed varied significantly with anti-WNV antibody positivity. The local-breed Dongola horses had a higher likelihood of being seropositive than the imported Argentine Criollo horses ($p=0.006$; OR = 2.51 [95% CI 0.78–8.16]). It was observed that 35.7% of tested sera were positive for anti-WNV neutralizing antibody (*i.e.*, PRNT₉₀ titer from 1:10 to 1:160). The sera that were negative by PRNT but positive by cELISA probably had antibodies that were hemagglutination-inhibiting or complement-fixing, but not neutralizing.

TABLE 2. INDEPENDENT ASSOCIATION BETWEEN STUDY VARIABLES AND ANTI-WEST NILE VIRUS ANTIBODY POSITIVITY AMONG THE STUDY HORSES IN SOUTHWESTERN NIGERIA

Variables	Number tested	Number positive (%)	Odds ratio (95% confidence interval)	p value
Location				
Ikoyi	79	66 (83.5)	1 ^a	
Ibadan	45	44 (97.8)	4.6 (0.58–36.79)	
Ajah	21	21 (100.0)	1.7E8 [†]	0.15
Sampling year				
2011	46	45 (97.8)	4.7 (0.59–37.61)	0.17
2012	99	86 (86.9)	1 ^a	
Horse breed				
Criollo	44	34 (77.3)	1 ^a	
Dongola	101	97 (96.0)	2.5 (0.78–8.16)	0.006*
Gender				
Male	29	26 (89.7)	1 ^a	
Female	116	105 (90.5)	9.5 [†]	0.89

1^a, reference group.

*Statistically significant at $p \leq 0.05$.

[†]Confidence interval (CI) was not computed by SPSS.

This observation could be due to stringency of the PRNT or nonspecific reaction of the horse sera with antigenically related flaviviruses in the cELISA. However, the identity of such flaviviruses is unclear, although Wesselsbron virus is known to infect horses (Weiss et al. 1956). None of the 145 horses had detectable anti-WNV IgM. This implies that the horses were infected at a sufficient time prior to sampling to allow anti-WNV IgM levels to decay but retain detectable anti-WNV antibodies, presumably IgG.

Horses stabled at Ajah were all positive for anti-WNV antibody, and this may have been associated with the high rainfall experienced in Lagos State. Evidence of obvious waterlogging at the time of sampling supports this and could enhance vector activity and spread of arboviruses at this location. Because the horses were housed under similar conditions, the higher levels of seropositivity observed for the local breed (Tables 1 and 2) compared to imported horses could be explained by a longer exposure time to indigenous mosquito vectors.

The overall high seroprevalence rate observed in this study is comparable to those reported in Chad (97.0%) and Senegal (92.0%), but much higher than reported for Cote d'Ivoire (28.0%), Democratic Republic of Congo (30.0%), Gabon (3.0%), and Djibouti (9.0%) (Cabre et al. 2006). In southwestern Nigeria, the only available study with which the present work can be compared was by Olaleye et al. (1989), in which a 71% seroprevalence of WNV antibodies was obtained using the complement-fixation test. From these investigations, it is apparent that southwestern Nigeria is endemic for WNV.

In conclusion, this study has shown that although there are no reports of West Nile disease suggesting recent infection in Nigerian horses, there is strong serological evidence of exposure to WNV, with high seroprevalence rates obtained at all sampling locations. These findings suggest that WNV activity had occurred in Nigeria in the years prior to the period of sampling.

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Author Disclosure Statement

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