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Serosurvey of West Nile virus in horses and detection of West Nile virus antigen in mosquitoes in Kaduna State, Nigeria

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West Nile virus (WNV) causes a mosquito-borne zoonotic disease of public health importance. The aim of this study was to determine the state of WNV infection in horses and detect the virus antigen in mosquitoes trapped in stables in Kaduna State Nigeria. The study was carried out in Kaduna State, Nigeria, and 368 horses were screened for the presence of antibodies against WNV using an IgG competitive enzyme-linked immunosorbent assay. Of the 368 samples tested, 331 (89.9%) were positive for WNV antibodies. Mosquitoes from the stables were tested for WNV antigen using a VectorTest kit, and of the 31 pools of adult mosquitoes tested, only 5 (16.1%) pools tested positive for WNV antigen. This finding showed that WNV infection has occurred in horses and that there is evidence of circulation of the virus by mosquitoes in Kaduna State, Nigeria.

Key words: *horses, mosquitoes, seroprevalence, West Nile virus*

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West Nile virus (WNV) causes a re-emerging mosquito-borne disease in humans, birds and animals [5, 6]. The virus was first reported in the West Nile District of Uganda in 1937 and was isolated from the blood of a febrile adult human [22]. WNV is a member of the Japanese encephalitis virus complex, which includes Japanese encephalitis virus, Saint Louis encephalitis virus and Murray Valley encephalitis virus, within the genus *Flavivirus*, family *Flaviviridae* [11]. It is also a disease that is commonly found in Africa, Europe, the Middle East and North America [28]. Infection caused by WNV is usually asymptomatic and is characterized by mild febrile illness to encephalitis with fatal outcome in humans and horses [10, 18]. The virus causes West Nile encephalitis, with initial symptoms usually being mild febrile illnesses, and its incubation period following mosquito transmission is about 3–15 days [17]. The virus is maintained by an enzootic cycle and it is transmitted mainly between birds and mosquitoes, with humans, horses and other animals serving as incidental and dead-end hosts. Infected mosquitoes harbor WNV in their salivary glands and are able to infect susceptible vertebrate hosts during feeding [19]. The global distribution of WNV mainly depends on the presence of susceptible avian reservoir hosts along with competent mosquito vectors, mosquito host preference and availability of susceptible hosts [14]. The presence of WNV throughout a large geographical region in Africa has been demonstrated in the past, before clinical infections were observed in some locations in Africa [7]. Seroprevalence of WNV has been reportedly high among horses in some parts of Sub-Saharan Africa [4]. Recently, high seroprevalence of the virus was reported in horses in Southwestern Nigeria [24].

There is a paucity of information on WNV in horses in Kaduna State, Nigeria. Providing evidence of this viral infection in horses and evidence of contemporary virus circulation would help justify vaccination of prized horses, such as thoroughbred race and polo horses, against the disease in Nigeria. Similarly, detection of the virus in mosquitoes is the major evidence needed to establish the possibility of animal and human outbreaks or occurrence of undetected outbreaks. This would help medical personnel to include WNV infections as part of the routine differential diagnosis of febrile illnesses in Nigeria. The aim of this study was to determine if WNV antibodies are present determine if WNV

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antibodies are present in horses and to detect WNV antigen in mosquitoes in Kaduna State, Nigeria.

The study was conducted in Kaduna State, Nigeria, which is located in the North West Zone of the country (longitude E006.5°−E008.6° and latitude N09.2°−N11.3°) [13]. The State is essentially agrarian, with about 75% of the population engaging in farming, and it also has potential with respect to the livestock industry [16]. The State has a strong traditional institution with emirs in Zaria and other major towns that keep horses. There are also military, police and polo horses in the State. This study was carried out in a selection of Local Government Areas (LGAs) of Kaduna State, namely the Sabon Gari, Zaria, Igabi and Kaduna North LGAs (Fig. 1).

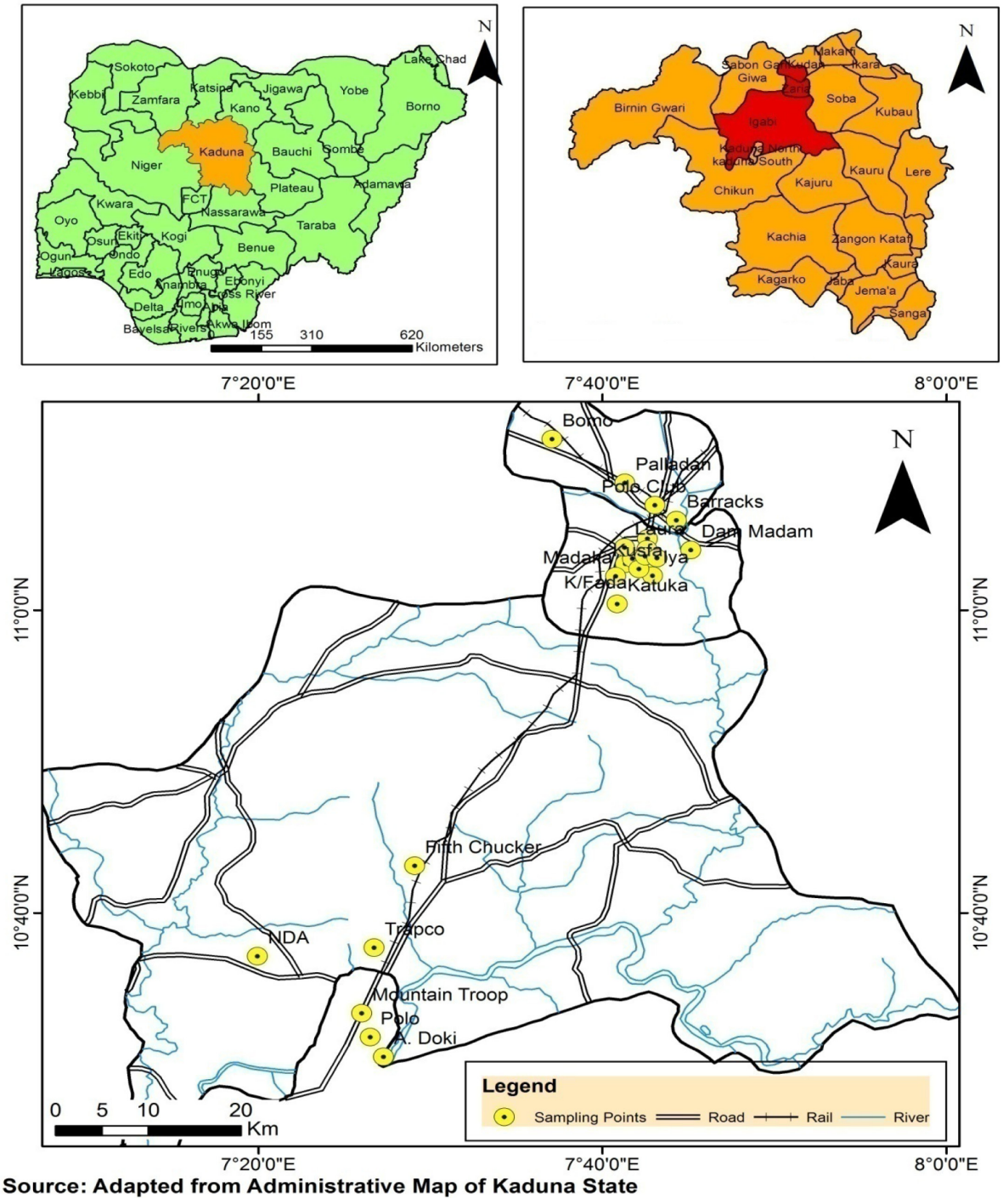

Fig. 1. Map of Kaduna State showing the sampling area.

This study was conducted as a cross-sectional study of horses and mosquitoes associated with horses in stables. Blood samples from horses were collected in February to April 2016. The study was conducted in a purposive selection of LGAs of Kaduna State known to have significant numbers of horses [16].

The sample size was determined according to the formula described by Thrusfield [25]:

$$N = \frac{Z^2 \, P_{exp} \, q}{d^2},$$

Where:

N was the sample size,

Z was the standard normal deviate for the 95% confidence interval (1.96),

P was the prevalence (90.3%) [24],

d was the desired precision (0.05), and

q was 1 − P (1 − 0.903=0.097). So,

$$N = \frac{1.96^2 \times 0.903(1-0.903)}{(0.05)^2},$$

$$N = \frac{34816 \times 0.903 \times 0.097}{0.0025},$$

n=134.6.

Ultimately, 368 samples were collected from horses from the selected LGAs of Kaduna State in order to make full use of the test kit.

Five millilitres of blood was collected aseptically from the jugular vein of apparently healthy horses using 18G needles. The samples were labelled and transported in a cold box to the laboratory, where they were centrifuged at 3,000 g for 5 min. The serum was harvested from each blood sample, labelled and preserved at −20°C until it was used for analysis.

Mosquito pools were collected from the stables where the horse blood samples were collected. A total of 775 adult female *Culex* mosquitoes were collected and aggregated into 31 pools containing 25 female *Culex* mosquitoes per pool. A CDC Miniature Light Trap (Model 512, John W. Hock Co., Gainesville, FL, U.S.A.) acquired from the Department of Biological Sciences, Ahmadu Bello University, Zaria, was used to trap adult mosquitoes in the horse stables in the selected LGAs of Kaduna State. The trap was left to operate from dusk to dawn. The trapped mosquitoes were emptied into well-labelled sterile sample bottles, preserved with silica gel and transported to the laboratory, as described by Mayagaya *et al*. [15]. The trapped mosquitoes were taken to the Entomology Laboratory of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, where they were sorted into species by visual observation using a simple light microscope to separate different species of adult mosquitoes using the physical characteristics of the head and thorax, in addition to the wing anatomy, markings on the hind legs and abdomen and nature of the palps [8, 21]. Adult female *Culex* species (*C. pipiens*, *C. univittatus* and *C. quinquefasciatus*) were selected for the detection of WNV antigen.

The experiment was performed in accordance with a protocol for the care and use of experimental animals and was approved by the Faculty of Veterinary Medicine Ethics and Research Committee at the Ahmadu Bello University, Zaria, Nigeria.

All serum samples were tested for anti-WNV IgG antibodies with a competitive enzyme-linked immunosorbent assay (cELISA; ID Screen West Nile Competition Multi-species, IDvet, Grabels, France). The protocol was carried out according to the manufacturer's instructions.

Thirty-one pools of adult mosquitoes were used for the detection of WNV antigen in mosquitoes using a VectorTest WNV Antigen Assay (VecTOR Test Systems, Inc., Thousand Oaks, CA, U.S.A.), and this was carried out according to the manufacturer's instructions.

The data generated were analysed using IBM SPSS Statistics version 20.0 to carry out a descriptive analysis of the distribution of the disease and variables. The Chi-square test was used to determine associations between demographic variables and occurrence of WNV infection. Values of $P \leq 0.05$ were considered significant. The odds ratio (OR) and 95% confidence interval were used to assess risk factors of WNV infection. Ethical clearance and approval of the study design and methodology were granted by the Ministry of Agriculture, Kaduna State, Nigeria.

The overall seroprevalence of WNV antibodies in horses was 89.9% in the 368 samples screened by cELISA. Out of the 100 female horses screened, 89 (89.0%) were seropositive, whereas 242 (90.3%) of the 268 male horses screened were seropositive. There was no statistically significant association ($P>0.05$) between the sex of the horses screened and the prevalence of WNV. Regarding the distribution of WNV in horses by age, the highest seroprevalence was found in horses greater than 20 years old (100%), followed by horses that were 16–20 years old (92.5%), and the lowest seroprevalence was found in horses that were 6–10 years old (86.9%). There was no statistically significant association ($P>0.05$) between horse age and the prevalence of WNV. Though there was no statistically significant association ($P>0.05$) between the breeds of the horses screened and the prevalence of WNV, the seroprevalence was highest in the Sudanese breed (94.7%), followed by the Talon (91.2%) and Arewa breeds (91.0%), and the lowest seroprevalence was found in the Argentine breed (85.9%). Based on location, the seroprevalence of WNV in horses was highest in the Kaduna North LGA (98.9%), followed by the Zaria and

Igabi LGAs (88.7% and 86.0%), and the lowest seroprevalence was found in the Sabon Gari LGA (78.9%). There was a statistically significant association ($P<0.05$) between the seroprevalence of WNV infection and the location of horses (Table 1).

A total of 775 adult female *Culex* mosquitoes were trapped in stables and pooled into 31 groups. Out of the 31 adult female *Culex* mosquito pools tested, only one adult female *Culex* mosquito pool (3.2%, 1/31) from Kaduna State tested positive for WNV antigen. The positive pool was one of 14 (7.1%, 1/14) adult female *Culex* mosquito pools obtained from the Zaria LGA. Doubtful results were obtained for four adult female *Culex* mosquito pools from the Sabon Gari LGA (23.1%, 3/13) and Zaria LGA (7.1%, 1/14). There were no WNV antigen reactions in the pools sampled from the Igabi and Kaduna North LGAs.

The overall seroprevalence of WNV antibodies in horses was 89.9% in the selected LGAs of Kaduna State, Nigeria. This suggests that the horses were exposed to the virus, and this could be attributed to the poor environmental management system adopted by some of the grooms in and outside the horses' stables and also to the wet and warm season during which the horses were sampled. Mosquitoes tend to be more abundant during the rainy and hot season, and transmission of the virus could be possible at this time. The seroprevalence of WNV infection in this study is comparable to those reported by previous researchers in Nigeria and in other countries. Sule *et al*. [24] reported a prevalence of 90.3% in horses in Southwestern Nigeria. Ezeifeka *et al.* [9] and Baba *et al.* [2] reported lower WNV prevalences in horses of 25.0% and 11.5%, respectively, in Nigeria. Similarly, low prevalences were described in reports from Djibouti (9.0%), Cote d'Ivoire (28.0%), Democratic Republic of Congo (30.0%) and Gabon (3.0%) [4]. In other parts of the world, two provinces of Pakistan had prevalences of 65.0%, as reported by Zohaib *et al.* [29]. Also, Ute *et al*. [27], Sadegh *et al*. [20] and Strahinja *et al*. [23] demonstrated low WNV prevalences of 13.5% in Ukraine, 2.8% in Iran and 28.6% in Northern Serbia, respectively. It is possible that these low prevalence levels are due to differences in the types and sensitivities of the tests used, such as the complement fixation test and microneutralization test. There was no statistically significant difference in seroprevalence based on sex in this study. Our results agreed with the previous work reported by Baba *et al*. [2], who also reported a higher prevalence in male horses in Nigeria. It has been reported that the development of neuroinvasive disease in humans due to WNV infection occurs more frequently among males. On the other hand, the risk of initial infection with WNV has not been found to be significantly higher among males according to serosurveys and studies among human blood donors [3, 26].

There was no statistical association between any of the age groups of the horses and WNV infection in this study. Age has been considered to be one of the risk factors for WNV infection due to suppression of immunity. Pradier *et al*. [19] reported that the incidence of neuroinvasive WNV disease and death is associated with increase in age. The lack of correlation between age and seroprevalence in the present study may suggest equal opportunities for exposure to mosquitoes in all age groups of the horses, as there was no preferential housing system for young or old horses in the stables

Although there was no statistically significant difference between the breeds of horses screened, Sule *et al*. [24] reported in a similar report that Dongola horses (Arewa horses) showed high seropositivity, whereas Ezeifeka *et al.* [9] reported 30.1% lower seropositivity in an Arabian cross (Talon breed) compared with other breeds in their work. These differences could be due to a long period of exposure to an indigenous mosquito vector [24]. In the present study, the seroprevalence was highest in Kaduna North, followed by Zaria, with the lowest seroprevalence found in the Sabon Gari LGA, and there were statistically significant differences in WNV seroprevalence among the locations of the horses screened.

Mosquitoes, which are the primary vector of WNV, play a major role in the transmission of the virus in birds, humans, animals and reptiles. This study detected WNV antigen in a pool of mosquitoes trapped in horse stables in Kaduna State. This suggests that there is ongoing WNV circulation in the State. Other researchers, Hidalgo-Martínez *et al*. [12],

Table 1. Seroprevalence ofWest Nile virus antibodies in horses in Kaduna State based on location

Location (LGA)	No. samples tested	No. samples +ve (%)	No. samples −ve (%)	Odds ratio	95% CI	*P* value
Zaria	150	133 (88.7)	17 (11.3)	Ref		
Igabi	100	86 (86.0)	14 (14.0)	1.274	0.597−2.717	
Kaduna North	95	94 (98.9)	1 (1.1)	0.083	0.011−0.636	0.003
Sabon Gari	23	18 (78.9)	5 (21.7)	2.173	0.715−6.608	
Total	368	331 (89.9)	37 (10.1)			

Ref, reference; +ve, positive; −ve, negative; CI, confidence interval.

reported one pool of mosquitoes positive for WNV antigen in Tabasco, Mexico. In comparison, Sule *et al*. [24] reported no WNV activity in mosquitoes in Southwestern Nigeria. Also, Baba *et al*. [1] reported no WNV positivity in 52 pools of mosquitoes tested in semi-arid zones of Nigeria even though they used RT-PCR, which is a more sensitive test as compared with the VectorTest.

Therefore, the difference in the number of positive samples between the present study and previous studies may be due to the place and/or time of sampling rather than the sensitivity of the testing methods.

In conclusion, the results obtained in this study indicate a high seroprevalence of WNV in horses in Kaduna State and also reveal evidence of WNV infection in mosquitoes in Kaduna State, Nigeria. Hence, there is a need to improve hygienic practice in and around horse stables. Public awareness campaigns should be carried out to raise public awareness of mosquitoes-borne zoonotic diseases, including WNV infection.

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