Evidence of West Nile virus in chickens and horses in Nigeria: results from a serosurvey

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Summary
West Nile virus (WNV) is an emerging arbovirus which affects humans and horses. A cross sectional study was carried out on 106 local horses in Kaduna and 78 domestic chickens in Federal Capital Territory. A total of 184 sera were screened for West Nile virus anti Pr-E antibodies using ID Screen® West Nile competitive enzyme linked immunosorbent assay. For the horses, an overall prevalence of 92.45% was recorded while domestic chickens had a preponderance of 7.69%. From our study, there was a statistical significant difference between the occurrences of WNV in stallions than mares with $p < 0.05$. Comparing the occurrence of West Nile virus between species, horses were more likely to be infected by West Nile virus than domestic chickens (OR 147). This is the first seroprevalence study investigating West Nile virus infection in domestic chickens in Nigeria. The presence of the antibodies indicates the widespread circulation and the potential risk of infection in humans and animals. In order to understand the epidemiology of West Nile virus infection in Nigeria, there is need for surveillance to be implemented in human and animal sectors.

Introduction

West Nile virus (WNV) is a vector-borne virus belonging to the Japenese encephalitis complex within the *Flaviviridae* family. The complex includes Japanese encephalitis virus (JEV), Saint Louis encephalitis virus (SLEV), Murray Valley encephalitis virus (MVE) and Usutu virus (USUV) (Fall *et al.* 2016). Most of these flaviviruses, such as WNV, tick-borne encephalitis virus (TBEV), or JEV, are considered as emerging zoonoses that pose serious public health threats to animals and man (Cardinale *et al.* 2017).

West Nile virus was first isolated from a febrile woman in Omogo region in the West Nile district of Uganda in 1937, from a set out survey to define the endemicity of yellow fever (Smithburn *et al.* 1940). WNV is chiefly transmitted by bite of infected *Culex* sp. mosquitoes. Birds are considered as a main reservoir host and migratory birds can play an important role in long distance viral dissemination (Hubalek and Halouzka 1999, Hayes *et al.* 2005, Ergunay *et al.* 2015). Humans and horses are known to be incidental hosts of WNV (Murray *et al.* 2010). Clinical symptoms of WNV infection may range from asymptomatic or mild influenza-like illness to severe neurological disease, which can be characterized by acute flaccid paralysis, encephalitis, and meningoencephalitis (De Fillet *et al.* 2012, Vilibic-Cavlek *et al.* 2014).

From the 1990s, West Nile disease outbreaks have emerged across various continents and WNV is now recognized as one of the most prevalent flaviviruses worldwide (Vilibic-Cavlek *et al.* 2014, Cox *et al.* 2015, Fall *et al.* 2016). In Nigeria, *Culex* species like *Culex pipens, Culex quinquefasciatus* have been reported in South West Nigeria (Motayo *et al.* 2016). This is a clear indicator that most of the vectors in Nigeria are present and may predispose both humans and animal population to the WNV infection. West Nile Virus has been reported in horses and humans by different authors in Nigeria. Olaleye and colleagues (Olaleye *et al.* 1989) reported a prevalence of 71% from horses in Southwest Nigeria, Sule and colleagues (Sule *et al.* 2015) recorded 90.3% in the same geopolitical zone while Baba and colleagues (Baba *et al.* 2013) documented a prevalence of 25% from humans in Maiduguri North East Nigeria.

Avian species have been categorized into four groups for the purpose of WNV surveillance: dead birds, trapped birds, captive and sentinel birds or domestic birds (Chintoutis *et al.* 2015). In the United

States of America and Italy, for decades, domestic birds have been used as living sentinels in arbovirus programs aimed at monitoring virus transmission (Komar *et al.* 2003b). West Nile virus surveillance using domestic birds has been documented in America, Europe and Australia (Chintoutis *et al.* 2015) and Africa (Fall *et al.* 2016, Amdouni *et al.* 2020). Chickens are frequently being used as sentinels for the surveillance of bird-transmitted arboviral encephalitides (Langevin *et al.* 2005, Blackmore *et al.* 2003). Furthermore, arbovirus surveillance systems based on testing of sentinel chickens provided evidence of WNV circulation prior to the occurrence of human cases (Healy *et al.* 2012). This study was therefore carried out to determine the presence of WNV infection serologically in domestic chickens and local horses in Nigeria.

Materials and methods

Ethical statement

This study was approved by the University of Abuja Ethical Committee and Animal Care Committee UAECAU/2019/01. Horses and domestic chickens were selected with the consent of their owners after they were briefed on the objective of the study.

Study area, design and sample collection

A cross sectional study was conducted with blood samples taken in 2019 from a total of 106 randomly selected local horses in three local government areas of Kaduna State with coordinate (10°30'36.7"N 7°25'01.6"E). The three local government areas were selected based on the concentration (Mshelia *et al.* 2012) of local horse presence of polo fields, and their unvaccinated status: Zaria LGA (11°03'40.6"N 7°42'20.6"E) (n = 40), Sabon Gari LGA (11°08'01.0"N 7°43'02.0"E) (n = 30) and Igabi LGA (11°08'01.0"N 7°43'02.0"E) (n = 36) Figure 1 and Table I. Similarly, blood samples from domestic chickens (n = 78) were collected from Federal Capital Territory (9°04'26.3"N 7°28'43.1"E) in commercially reared chickens (Isa Brown) (n = 40) and free range chickens (n = 38) in Kuje Area Council (8°52'49.0"N 7°13'37.7"E) to determine the spread of WNV. Blood samples were aseptically taken by jugular venipuncture and were allowed to clot and centrifuged at 10,000 g for fifteen minutes to allow for proper separation of serum from the clotted blood. Sera were harvested using a sterile pipette into 2 ml cryovial tubes, labeled and stored at - 80 °C at the National Veterinary Research Institute Laboratory, Vom, Nigeria for sample analysis.

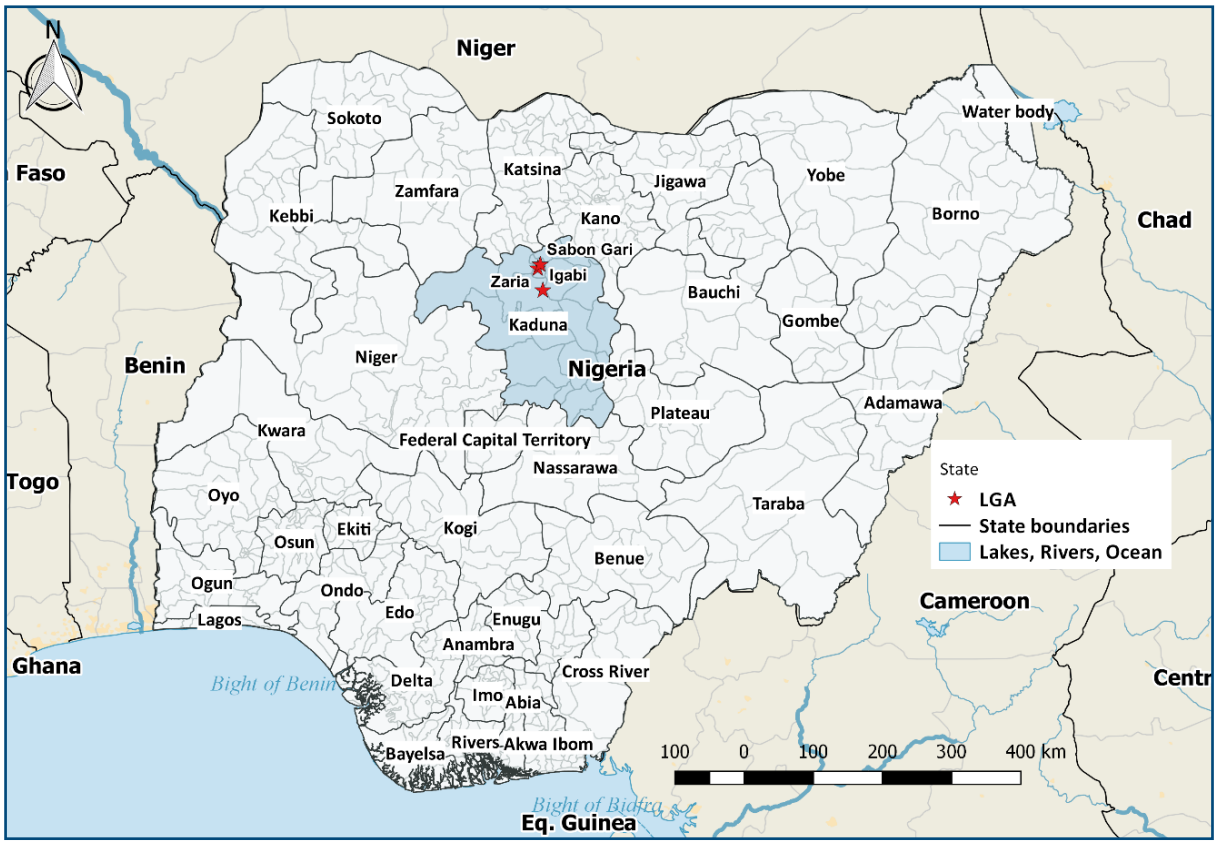

Figure 1. *Map of Nigeria with Kaduna state highlighted and sampling local government areas in red.*

Detection of WNV specific antibodies

Serum samples (n = 184) were screened for the presence of anti-WNV IgG antibodies using the ID Screen® West Nile competitive ELISA kit (IDVet, Grabels, France) that detects WNV anti-Pr-E antibodies, according to the manufacturer's instructions. Absorbance values were read at 450 nm wavelength, using a microplate spectrophotometer (Thermo Scientific Multiskan® EX; Thermo Fisher Scientific, Waltham, Massachusetts, USA). A presumptive positive diagnosis was made when the test samples produced an optical density (OD) less than or equal to 40%, they were considered as doubtful when the OD was between 40%-50%; and negative when the OD was greater than 50%.

Data analysis

Results obtained from serological tests were subjected to analysis by SPSS version 20.0 statistical packages for descriptive statistics. Chi square test and Fisher's exact test were used to test for association between categorical variables; p value of less than < 0.05 was considered significant. Odd ratio was used to test for strength of association.

Results

Prevalence of WNV antibodies in local horses in selected local government areas of Kaduna State

A total of 106 local horses were sampled with an overall prevalence of 92.45%. Zaria local government area recorded the highest prevalence (38/40; 95%), followed by Igabi local government area (34/36; 94.44%) and Sabon Gari LGA (26/40; 86.67%) (Table I). Horses in the sampled areas were divided into three age groups. The first group included animals 1-5 year old, the second included animals between 6 and 10 years, and the third animals aged between 11 and 15 years. The prevealence found in the first group was 80% (12/15), 96.83% (61/63) was the prevalence found in the second group while in the third group it was 89.29% (25/28). All males (100%; n = 94) were found positive to the WNV c-ELISA, while in only 66.67% of the sampled mares (n = 12) WNV antibodies were detected. There was a statistically significant difference (p < 0.05) between the occurrences of WNV in stallions and in mares (Table I).

Table I. *Distribution of West Nile Virus antibodies in local horses in three local government areas of Kaduna State according to demographic characteristics.*

Variables	No. Sampled	No. Positive	% Prevalence (95% CI)	*p* value
Sex				
Male	94	94	100 (NaN - Infinity)	0.0001*
Female	12	8	66.67 (37.69-88.39)	
Age (years)				
1-5	15	12	80 (54.65-94.65)	0.065
6-10	63	61	96.83 (89.91-99.46)	
11-15	28	25	89.29 (73.55-97.20)	
Location (LGA)				
Zaria	40	38	95 (84.45-99.15)	
			86.67 (70.90-95.62)	0.365
Sabon Gari	30	26	94.44 (82.84-99.06)	
Igabi	36	34		

*NaN-Infinity = Not a number.

Prevalence of WNV antibodies in chickens in Kuje local government area

A total of 78 chickens were sampled in Kuje local government area of Federal Capital Territory with an overall WNV prevalence of 7.69%. Free range chickens recorded a prevalence of 5.26% (2/38) while in exotic chickens (Isa Brown hybrid chicken) the prevalence found was 10% (4/40/) (Table II).

A total of thirty eight (38) free range birds where sampled. All cocks (n = 4) were negative. A prevalence of 5.88% was detected in the 34 hens (Table II).

Comparison of WNV antibodies among local horses and chickens in Kaduna and FCT

From the sampled horses, 92.45% (98/106) prevalence was recorded in Kaduna State while 7.69% (6/78) was recorded from chickens in the Federal Capital Territory. In this study the WNV prevalence found in horses was much (p < 0.05) than that found in chicken. Also, horses were 147 times more likely to be infected by WNV than chicken (OR 147) (Table III).

Discussion

The present study highlighted the circulation of West Nile specific IgG antibodies using competitive enzyme linked immunosorbent assay (c-ELISA) in Nigerian local horses and chickens (exotic and free range chickens) in Kaduna and Federal Capital Territory Abuja, respectively. A prevalence of 92.45% was recorded in horses. The result of this study was similar to other reports of WNV in horses in Tchad 90.3% or Senegal 92.5%. Conversely it was much higher than that found in Cote d'Ivoire 28%, Congo Democratic Republic 30%, Gabon 3% and Djibouti 9% (Cabre *et al.* 2006, Sule *et al.* 2015). The high

Table II. *Distribution of West Nile Virus antibodies among domestic chickens in Kuje Area Council, Federal Capital Territory.*

Variables	No. Sampled	No. Positive	% Prevalence (95% CI)	p value
Breed				
Local	38	2	5.26 (0.89-16.32)	0.4326
Exotic	40	4	10 (3.26-22.38)	
Sex (Local)				
Male	4	0	0	0.618
Female	34	2	5.88 (1.00-18.10)	
Sex (Exotic)				
Male	0	0	0	Not determined
Female	40	4	10 (3.26-22.38)	

Table III. *Comparison of WNV antibodies among local horses and domestic chickens in the study areas.*

Species of animal	No. sampled	No. positive	Proportion (%) (CI)	95% CI	*p* value
Horses	106	98	92.45	86.17-96.43	0.0001
Chickens	78	6	7.69	3.18-15.31	
Total	184	104	56.52	49.99-63.24	

prevalence of WNV recorded in this study could be due to factors such as tranboundary movement of horses from neighboring African countries into Nigeria and climate change. The vegetation of Kaduna is Sudan Savannah with short trees, shrubs and grasses and relatively low rainfall that provide conducive conditions for the proliferation and adaptations of vectors that are capable of transmitting the virus to horses which corroborate with the studies conducted by Hubalek and Halouzka and Chancey and colleagues (Hubalek and Halouzka 1999, Chancey *et al.* 2015). Kaduna State is characterized by high temperature which can cause an upsurge in growth rates of vector populations, decrease the interval between blood meals, shorten the incubation time from infection to infectiousness in mosquitoes, accelerate the virus evolution rate and increase virus transmission efficiency (Paz 2015).

Local horses are used for different purposes ranging from sports (horse racing or polo), religious festivals and some are slaughtered for meat. horses are transported to different part of the country during competitions with the possibility of of introducing vectors along with them into naïve population further creating a complex dynamic in the transmission of WNV. Also, less attention is given to the health and management of local horses as they are believed to be resistant to disease pathogens. This is in sharp contrast with the exotic horses which are well groomed and vaccinated or treated against various infectious diseases including WNV from their country of purchased. Studies have documented that WNV infection risk increases during peak mosquito activity seasons (August-October) in temperate zones of the Northern hemisphere, however, this observation is different in tropical and subtropical regions where disease epizootic patterns does not conform to what is obtainable in temperate regions (Zohaib *et al.* 2015). Horses in the age range of 6-10 years were found to record the highest prevalence of WNV antibodies. This could be attributed to the fact that they must have spent longer time in their stables thereby been exposed to several facilitating risk factors associated with the disease. This is contrary to the studies conducted by Zohaib and colleagues (Zohaib *et al.* 2015) in Pakistan where antibodies to WNV decreased with age. The significant difference observed between sexes in our study where stallions recorded more seropositivity may be attributed to the availability of more stallions found in the location during sampling than mares and their use in ceremonial and sporting activities in northern part of the country. This is contrary to the studies of Sule and colleagues (Sule *et al.* 2015) in Southwest Nigeria where mares recorded a higher seropositivity than stallions.

Birds are generally known to be host of WNV and other flaviviruses (Furlong *et al.* 2023). Overall prevalence of 7.69% was recorded among domestic chickens in our study. A similar study was carried out by Yapici and colleagues (Yapici *et al.* 2012) among domestic chickens with no seropositivity recorded. Several authors have worked on WNV infection in chickens with varying prevalence 0.63% by Komar and colleagues (Komar *et al.* 2003a) while Le Francois and colleagues (Le Francois *et al.* 2006) recorded 1.7% and 0.6%, respectively, which were all lower than our study. However, Jozan and colleagues (Jozan *et al.* 2003) had 69% preponderance which is way higher compared to the recorded prevalence.

From our study, exotic chickens had a higher preponderance than the free range chickens. The low preponderance of WNV antibodies observed in free range chickens as compared to exotic chickens could be linked to location. Most of the poultry farms are sited far away from human settlements with only farm workers residing in the farm. This encroachment of the natural habitat of mosquitoes may have played a role in the difference between breed preponderance of the WNV infection. Exotic chickens are exposed several vectors such as mosquitoes and soft ticks which can be found in the crevices of walls and fruit bats that roost on trees and roofs of poultry pens and farm houses. Thereby, predisposing poultry workers in poultry farms and markets (those that sell and dress chickens) to WNV infection. On the other hand, free range chickens wander occasionally into nearby bush to scavenge for feed and return home for shelter around their

pens or some sleep outside by perching on trees, thereby exposed to adverse weather conditions and mosquitoes. At least, there is an effective mosquito control around homes where these chickens reside.

From the free range chickens, hens were the only chickens that tested positive. Hens are mostly kept in rural areas with only few cocks that have good reproductive pedigrees are left to stay longer in the population. Cocks are either sold to meet immediate economic challenges or consumed during festivals and ceremonies.

Horses and chickens seropositivity were compared. Horses were highly likely to be infected with WNV than chickens. This was attributed to the different age and exposure time to the WNV. Vitek and colleagues (Vitek *et al.* 2005) remarked that horses experience deleterious effect when they are infected with WNV infection. However, horses are considered incidental host because they do not develop sufficient viremia to infect mosquito vectors. Conversely, morbidity and mortality among avian species varies (Vilibic *et al.* 2014), birds are thought to play a vital role as host for WNV life cycle due to the fact that they develop viremia adequately enough to infect mosquitoes.

In conclusion, this study was able to demonstrate the presence of WNV antibodies in local horses and domestic chickens (exotic and free range) in Nigeria, to the best of our knowledge; this is the first report in chickens. Since avian species play an important role in WNV life cycle, its close interaction with humans poses a risk of zoonosis as some of the febrile illness in man can be misjudged as malaria in communities with poor diagnostic capabilities. There is need to assess the clinical impact of WNV infection on humans, equine and domestic chicken populations. Further molecular studies are needed in order to characterize the WNV in various livestock and human populations. This will be able to rule out any cases of cross reactions with other arboviruses.

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