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West Nile Virus Sero-Reactive Animals Reveal Potential Zoonotic Threat In Nigeria

Olanrewaju Eyitayo Igah[1*], Seyi Joshua Oyetunde[1], David Oludare Omoniwa[2], Emmanuel Richard Edeh[2], Olayinka Asala[1], Ismaila Shittu[1], Clement Meseko[1].

[1]National Veterinary Research Institute,[2] Faculty of Veterinary Medicine, University of Jos. *Corresponding author: Email: lanre.igah@gmail.com; Tel No: +2348062255816

ABSTRACT

West Nile Virus (WNV) is a zoonotic mosquito borne arbovirus that causes encephalitis in horses and human. It is a pathogen of public health importance globally. Data on WNV epizootics in Nigeria is limited, necessitating seroprevalence studies in animals. In this study, 118 sera from Polo horses and 66 archival sera from 31 horses, 10 chicken, 15 rabbits and 10 dogs in Northern and South-Western Nigeria were screened for WNV using West Nile multi-species competitive ELISA. The overall seroprevalence of WNV was 78.26% (n=144). In horses, 93.28% (n=139) of the 149 horse sera were positive, all the 10 chicken samples tested were negative to WNV, only 1 (6.67%) of the 15 rabbits sera tested positive and 4(40%) of 10 dog sera tested was positive.

The lack of sero-reactivity in domestic chickens in this study does not suggest a low incidence rate in wild birds that are known reservoirs. Sero-reactivity in rabbits and dogs indicates their exposure and susceptibility to WNV and therefore requires further investigation. The high sero-reactivity recorded in horses reiterates the important role they play in WNV epizootics which underscores the public health risk associated with WNV at the human-animal interface in Nigeria.

Keywords: West Nile Virus, Serology, Horses, Dogs, Rabbits, Chicken.

INTRODUCTION

West Nile virus (WNV) is a zoonotic, mosquito borne arbovirus belonging to the genus *Flavivirus* and the family *Flaviviridae* (Calisher & Gould, 2003). Variations in genomic sequence data reveals the existence of multiple WNV lineages (Donadieu et al., 2013). Lineage 1 and 2 identified as major lineages, were isolated in Africa while other minor lineages 3 and 4 were isolated separately in the northern hemisphere with more

lineages being reported in Africa (Fall et al., 2017; Rizzoli et al., 2015). WNV alongside St. Louis encephalitis virus (SLEV), Murray Valley encephalitis virus (MVEV), and Usutu virus (USUV) are members of the Japanese encephalitis virus (JEV) serogroup of flaviviruses (Mackenzie et al., 2002; Poidinger et al., 1996) which are the leading scauses of arthropod borne encephalitis in animal hosts (Murray et al, 2010). WNV is now considered a major aetiologic agent of viral encephalitis globally (Chancey et al., 2015).

Prior to 1999, WNV was restricted to Africa, but now causes disease globally and responsible for the upsurge of encephalitis in horses and man, making it a pathogen of global importance and a serious cause for concern due to its enzootic and epizootic capacity (McVey et al., 2015; Michel et al., 2019; Petersen et al., 2013; van der Meulen et al., 2005). For 86 years since the first detection of WNV in Uganda, where it was named after the West Nile River (Smithburn et al., 1940), the incidence, virulence and global distribution of the virus has increased and may have been aggravated by climate change, flood and vector abundance (Khasnis & Nettleman, 2005).

The zoonotic spread of the virus occur between aviphilic mosquito vectors and avian hosts (amplifying host) (Murray et al., 2010) with a sporadic enzootic spillover to humans and horses (Chancey et al., 2015). The likelihood of infection rises with age among horses in which they are endemic (Olufemi et al., 2021). The mosquito vectors are abundant and widely distributed in nature (Chancey et al., 2015). Moreso, infected mosquitoes show a characteristic feeding pattern which in addition to several human behaviors and interaction with the vector, results in bites that increases the chances of infection by WNV (Sule et al., 2018). West Nile sero-reactivity has been reported with about 55% of cases occurring in tropical and sub Saharan Africa during epidemics. This is attributed to the high level of exposure of humans to WNV as a result of existence of abundant human biting mosquitoes compared to the low incidence rate reported in temperate North America and Europe (Dauphin et al., 2004; Sule et al., 2018).

Other non-vector route of transmission of West Nile fever include oral means in cats, birds and other vertebrates(Austgen et al., 2004; Komar et al., 2003; Miller et al., 2003). Human infection can also occur through organ transplantation (Iwamoto et al., 2003), blood transfusion (CDC, 2002b), intrauterine and via breast milk (CDC, 2002). The WN virus is responsible for outbreaks of multiple viral encephalitis in humans, and is classified as the most widely spread flavivirus (Saxena et al., 2017). One (1) out of 4 infected persons develop West Nile Fever accompanied with varying clinical severity and below 1% of infected persons develop CNS signs such as encephalitis, meningitis and flaccid paralysis with 10% case fatality (Chambers & Diamond, 2003; Mostashari et al., 2001) . The clinical presentation of WN infection, the zoonotic origin, and the potential for wide and transboundary spread makes it a re-emerging zoonotic disease of public health importance. In South Africa, WNV have been reported in animals other than horses (Cattle, Buffalo, Dog, Deer, Giraffe, Goat, Lion, Sheep and Antelope with percentage prevalences of 2.2%, 1.9%, 4.6%, 33.3%, 9.1%, 9.1%, 11.1%, 2.2% and 7.1% respectively), 0% prevalence was also reported in birds in the same study. (Steyn et al., 2019). Birds, including migratory species and poultry develop sufficiently high and prolonged viraemia enough for mosquitoes to be infected

during feeding. The avian species are therefore regarded as an essential components of the life cycle of WNV. Thus migration of wild birds allows the spread of WNV into new territories and local ecological factors including interaction at the human-animal interface contributes to the epidemiology of WNV (van der Meulen et al., 2005). Despite this apparent risk and propensity for infection, data on WNV in Nigeria is scanty. This study therefore seeks to further investigate sero-reactivity of West Nile Virus in selected location and animals in Nigeria as part of One Health baseline data on the status of re-emerging zoonoses at the human-animal interface.

MATERIALS AND METHODS

Sera for this study were obtained by convenience utilizing an opportunistic sampling during the annual Jos International polo tournament that was held between 26th December 2021 to 2nd January 2022 in Jos, Plateau State. Archived sera from Horses, Chicken, Rabbit, and Dog obtained from Plateau state, Ondo state , Plateau state and Bauchi state were also included in the serological study. (Fig.1).

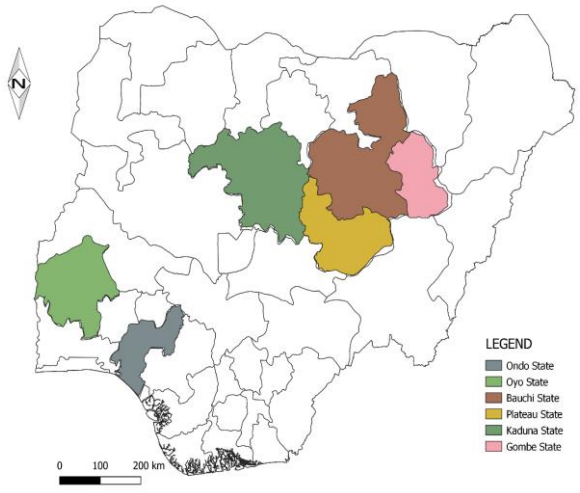

Fig.1: Map of Nigeria Showing sampled states.

About 3-5ml of blood were collected from the animals into sample bottles and sera separated after clotting. The samples were transported in cold chain to the Regional laboratory of the National Veterinary Research Institute, Vom and stored at -20^{0}C until utilized.

Serology

To estimate the seroprevalence, ID Screen® West Nile Competition multi-species ELISA kit was utilized. The assay was conducted on the sera to detect anti-pr-E antibodies according to the manufacturers recommended protocol. The optical density of samples was measured at 450 nm and the sample was considered positive if the sample/negative control percentage (S/N%) was ≤ 40% and negative if S/N% was > 50%.

Data Analysis

Results were presented in tables, Percentage (%) prevalence was calculated as

Number of Positive/total number of samples^{x}100

RESULTS

Of the 184 sera analyzed, 5.4% were chicken sera, 8.2% were from rabbit, 5.4% from dogs and 81% were sera from horses. Results from the serology revealed an overall sero-prevalence of 78.26% for WNV as shown in Table 1. Out of the 15 rabbits sera tested, only 1 (6.67%) tested positive. Out of 10 dog sera tested, only 4 (40%) tested positive, while 139 (93.28%) of the 149 (93.28%) horse sera tested positive as shown in Table 2

Table 1. Serological Detection of West Nile Virus

S/N	Location	Species	Number of samples	Positive samples	Percentage positive (%)	Total prevalence
1	Plateau (Polo horses)	Equine	118	110	93.22	
2	Plateau (Jos)	Equine	5	5	100	
3	Oyo	Equine	8	8	100	
4	Gombe	Equine	3	2	66.67	
5	Kaduna (Zaria)	Equine	5	5	100	
6	Bauchi	Equine	10	9	90	
7	Plateau (Vom)	Avian	10	0	0	
8	Ondo	Rabbit	15	1	6.67	
9	Plateau (Jos)	Canine	5	0	0	
10	Bauchi	Canine	5	4	80	
		Total	**184**	**144**		**78.26**

Table 2. Prevalence of West Nile Virus by Species

S/N	Location	Species	Number of samples	Positive samples	Total prevalence
1	Plateau (Polo horses)	Equine	118	110	
2	Plateau (Jos)	Equine	5	5	
3	Oyo	Equine	8	8	
4	Gombe	Equine	3	2	
5	Kaduna (Zaria)	Equine	5	5	
6	Bauchi	Equine	10	9	
		Total	**149**	**139**	**93.28**
7	Plateau (Vom)	Avian	10	0	
		Total	**10**	**0**	**0**
8	Ondo	Leporine	15	1	**6.67**
		Total	15	1	
9	Plateau (Jos)	Canine	5	0	
10	Bauchi	Canine	5	4	
		Total	**10**	**4**	**40**

DISCUSSION

West Nile Disease is an important zoonoses of which horses are identified as important sentinels (Venter et al., 2017). Seroprevalence study in horses living in close proximity to human settlements could predict the likelihood of occurrence of an outbreak in human. This study revealed the detection of WNV IgG antibodies in horses, rabbits and dogs implying a previous exposure and circulation of the virus within the study area. Since animals are not vaccinated against WNV in Nigeria, the antibodies detected in this study are likely due to previous natural

infection with WNV, suggesting an existing enzootic circulation of the virus between the mosquito vectors and animal hosts requiring further investigation.

The high sero-reactivity in horses and dogs in this study further reiterates the endemic nature of the disease and also an indication of the possibility of human exposure to the infection as a result of their ecological relationship; this depicts an existing zoonotic threat as with many infectious diseases in Nigeria (Meseko et al., 2021). Report from Africa shows 35% (1998 of 5746) of equids (horses, donkeys and mules) screened for WNV tested positive with seroprevalence ranging from 17.4% to 90.3% in different African countries (Olufemi et al., 2021). Prior studies in Nigeria has revealed a widespread distribution and exposure of human to the disease (Abdullahi et al., 2020) though clinical cases frequently go undiagnosed and undetected possibly due to ignorance of the zoonotic capability of the diseases and the existence of its circulation among animals including pets and livestock.

Be that as it may, sera from wild birds were not included in this study and sera from domesticated birds shows no sero-reactivity, but the abundance of wild birds in Africa in the migratory routes through Nigeria (Meseko et al., 2018) alongside the aviphilic mosquitoes completes the requirement for the enzootic circulation of WNV evidenced by the high sero-prevalence in horses and dogs observed in this study.

The lack of seropositivity in domesticated chickens from this study could be due to lack of contact with wild birds that are known reservoirs and also corroborates that of Steyn et al, (2019) who reported similar prevalence in birds.

Though, specific serodiagnosis of WNV is often hindered by cross reactivity with other flaviviruses, this limitation does not negate the existence and circulation of this virus and other flaviviruses in Nigeria creating a potential for zoonotic spillover. This calls for further work to expand our understanding of the existence of zoonotic circulation of the virus in the country, to ensure its inclusion as a differential on presentation of clinical signs of the disease in human and at the human-animal interface.

CONCLUSION

WNV is endemic to Africa, its persistent emergence and re-emergence in Africa pose significant health threat to both man and animals. The sero reactive evidence revealed in this study especially in horses which act as sentinel for the West Nile disease underscores a necessity to protect human from WNV through mosquito bite prevention and mosquito control.

Due to widespread distribution of WNV and its mosquito vectors, an epidemiological investigation system should be put in place to ensure early detection of the disease in sentinel animals (mammals and birds) so as to prevent the occurrence of a spillover event.

A limitation of this study was that Neutralization test which is most reliable but however time-consuming and requiring a BSL-3 facility was not conducted to specifically confirm WNV.

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