Original Research Article

ATTEMPTED DETECTION OF WEST NILE VIRUS FROM WILD AND PERIDOMESTIC BIRDS WITHIN IBADAN METROPOLIS IN NIGERIA

ABSTRACT

A team of researchers reported detection of West Nile virus (WNV) in the faeces of experimentally-infected wild birds after being experimentally infected with the virus subcutaneously. This necessitated the need to investigate the potential transmission of WNV through faeces in wild and peridomestic birds in Nigeria even though the virus is an arbovirus commonly transmitted by mosquito. To confirm the data, on hundred and ten (110) wild and peridomestic birds were screened for the presence of WNV using reverse-transcriptase polymerase chain reaction method was used for amplification of the viral RNA. The birds were drawn from six locations in Ibadan, Oyo State, Nigeria within a period of 18 months Detection of WNV was made with 5% agarose gel electrophoresis. However, we failed to detect WNV in these samples.

INTRODUCTION

Wild and peridomestic birds have been implicated in the transmission of some infectious diseases, acting either as reservoirs or vectors for the causative agents in the transmission of many viruses (Jacob *et al.*, 2011). Birds can acquire or transmit viral infections via vertical or horizontal modes of transmission (Strauss and Strauss, 2008). Horizontal transmission could be venereal – from a vertically infected male directly to a female vector – or oral – feeding on an infected host/carrier of the virus or virus-contaminated foods or drinks (Strauss and Strauss, 2008; Weaver and Reisen, 2010). Arboviruses are known to employ mosquito-bird interaction in their transmission cycles (Strauss and Strauss, 2008), and West Nile virus (WNV) is one of the most known arboviruses.

West Nile virus belongs to the genus Flavivirus in the family Flaviviridae. It is classified as a mosquito-borne Flavivirus, and further classified within this group with the neurotropic viruses. WNV infects a wide range of vertebrates, with birds as the major hosts and vectors for trans-boundary transmission, amplification, and reservoir. According to CDC (2009), 326 birds have been associated with the virus, either by isolation or detection of its neutralising antibodies. Migratory water birds such as herons and egrets are involved in the movement of WVN into new areas, serving as reservoir and amplification hosts (Rappole *et al.,* 2000; Mackenzie *et al.*, 2004; Reisen *et al.,* 2009) while the viruses are being transmitted by multiple *Culex* species of mosquito (Reed *et al.*, 2003; CFSPH, 2009; Pfeffer and Dobler, 2010). Peridomestic birds such as House Finches (*Carpodacus mexicanus*) and House Sparrows (*Passer domesticus*) have also been heavily linked with the spread of WNV and St. Louis encephalitis virus (Gruwell *et al.*, 2000), acting as reservoirs and sometimes exhibiting pathological symptoms of the infection too. WVN has also been isolated from pigeons (Weber, 1979; CSFPH, 2009; Weaver and Barret,

UNDER PEER REVIEW

2004; Komar and Clark, 2006), and persistent antibodies have been found in doves and pigeons in a set of studies (Allison *et al.*, 2004; Gibbs *et al.*, 2005). Other implicated birds include ducks, geese and mallards (Reed *et al.*, 2003; CFSPH, 2009) and, hawks and eagles (Kuno and Chang, 2005; CFSPH, 2009).

Arboviruses are mainly transmitted via a host-vector-host cycle, usually employing a biological mode of transmission involving the virus replicating within an arthropod host before transmission (Weaver *et al.*, 1997). In a review carried out by Kuno and Chang (2005), it was reported that non-biologic transmission mechanisms are also observed in arboviruses, of which direct transmission is one of such methods in which faecal matter was included. Alexander (2000) reported that spread from bird to bird appears to occur as the result of either inhalation of excreted droplet particles or the ingestion of infective material such as faeces. These reports indicate that faecal droppings of infected birds, both symptomatic and asymptomatic, are potential sources of infection for viruses shed in birds' faeces. A note of public health concern is that most birds implicated are not only wild birds whose natural habitat are far away from urban population, but also peridomestic and domesticated wild birds which lives in close proximity to human population, hence increasing the chances of transmission of these viruses (Hatch, 1996; Alexander, 2000). While it has been reported that arboviruses can be transmitted through ingesting of substances contaminated by faeces of infected hosts (Strauss and Strauss, 2008), Kipps *et al.* (2006) demonstrated this by isolating and detecting WNV in the faeces of American and fish crows which had been experimentally infected with the virus through subcutaneous inoculation. The authors reported that although faecal shedding of WNV by crows indicates a potential for direct transmission of WNV through contact with faeces, faecal–oral transmission among crows in the wild is unknown. They also reported that the role of viral shedding in WNV transmission to birds or other vertebrates requires further research and that no studies have not evaluated the quantity of virus or conditions necessary to infect humans or other primates through contact with WNV-infected faeces.

Therefore, this study aims to investigate the presence of WNV in the faeces of the wild and peridomestic birds within a metropolis in Nigeria in order to ascertain the potential for transmission of the virus through faecal-oral route naturally.

UNDER PEER REVIEW

MATERIALS AND METHODS

Study Population and Sites: A total of 110 cloacal swabs (n = 60) and faeces (n = 50) of identified wild and peridomestic birds from the families Columbidae , Psattaculidae, Anatidae, Ardeidae, Ploceidae, Phansianidae and Accipitridae were collected as presented in Table 1. Samples were randomly collected across Ibadan city by selecting representative samples among the target population. The birds were selected according to their species or the families they belong to, and they include healthy ones (n = 87) and some exhibiting symptoms of illness (n = 23). Samples were collected from six different locations within Ibadan metropolis as presented in Figure 1. Domestic birds that were reared and sold at the population sites were excluded. Also, suspected birds that are not within Ibadan city were excluded.

Sample Collection: Sterile swabs were used to collect swabs from the anus /cloacae of large birds. Swabs of fresh faeces were taken from birds from free ranges (egrets/herons, pigeons/doves and village weaver), from those whose anuses were not wide enough or whose owners refused cloacal swabs (lovebirds, parakreets and parrots), and from potentially dangerous birds (wild geese and hawks/eagles, buzzards). Samples collected were transported in transport medium to the laboratory, where they were stored at -20 ^{o}C until analyses**.**

Detection of virus: Detection of suspected virus was done using reverse-transcriptase polymerase chain reaction methods. For RT-PCR analysis, RNA was extracted from 140 ml of PBS-diluted faecal supernatant using Jena Bioscience viral RNA extraction kit according to the manufacturer's recommended procedure, and eluted with 60 ml sterile water. cDNA Synthesis and PCR amplification was carried out as described: Reverse transcription was carried out using 1ml RNA, 0.2 µl of each primer, 4µl RT Buffer (SCRIPT), 1 µl dNTP mix, 1 µl DTT stock solution, 1 µl RNase Inhibitor, 0.5 µl Reverse Transcriptase (SCRIPT) and RNase-free water, added up to make up a total volume of 20 µl. The Reaction Mix was incubated at 50 ^{O}C for 10 min, followed by a further incubation at 50 ^{O}C for 30-60 mins. The mixture was heated to 70^{O}C for 10min to inactivate the reverse transcriptase. 2 units of DNase-free RNase was also added and incubated at 37 ^{O}C for 20 min to remove RNA. The cDNA synthesized was now used as template to synthesize the second-strand using polymerase chain reaction and stored at -20 ^{O}C. For amplification, each PCR reaction contained 2 µl cDNA template, 3 µl each primer, 2.5 µl Taq Mix and 2.0 µl Nuclease-free water, in a total volume of 12.5 µl. The primers used in amplifying E region (encoding the envelop protein) of the WV viral genome was reported in Johnson *et al.* (2001). First-stage primer sequences, amplifying a 445-bp region: 1401: 5'-ACCAACTACTGTGGAGTC-3', and 1845: 5'-TTC-CATCTTCACTCTACACT-3'. Nested primers amplifying a 248-bp region were 1485: 5'-GCCTTCATACACACTAAAG-3'and 1732: 5'-CCAATGCTATCACAGACT-3' Thermocycling conditions using a 9700 model thermocycler (Applied Biosystems) are as follow: Taq Activation (94^{o}C for 3mins); Template Denaturation (94 ^{O}C for 30 secs); Annealing (50 ^{O}C for 30secs); Template Elongation (68 ^{O}C for 30 secs); Final Elongation (72 ^{O}C for 7 mins). The expected amplicons sizes for first round and second round (nested) PCR are 464bp and 278bp

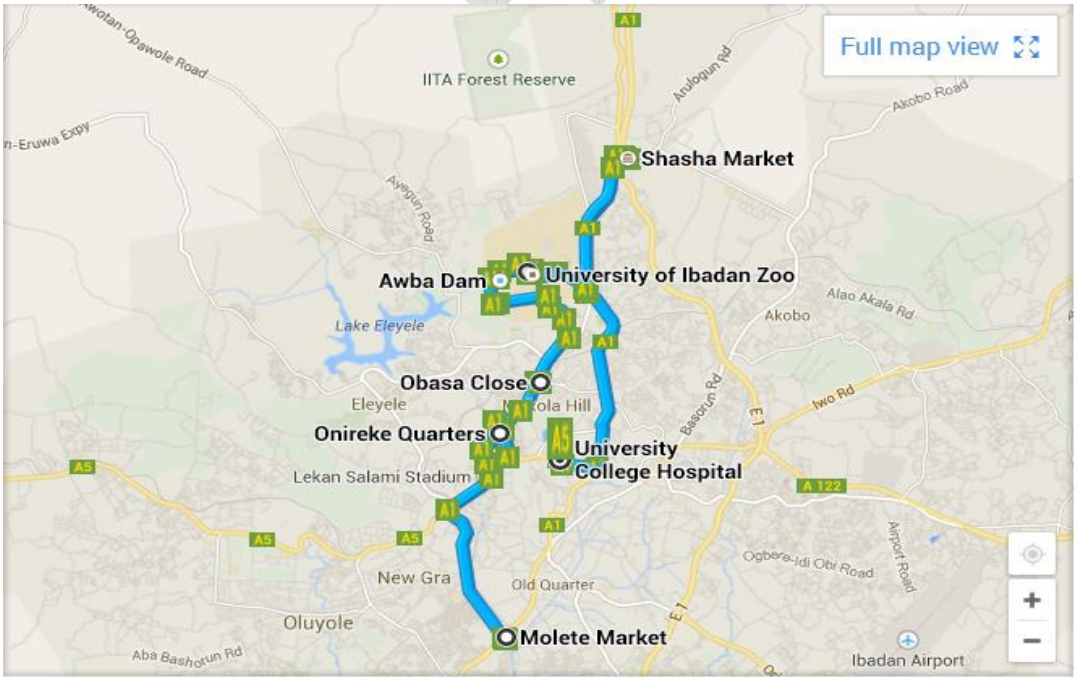
UNDER PEER REVIEW

98 respectively. Amplicons were analyzed using 3% agarose gel electrophoresis followed by ethidium
99 bromide staining and UV visualization

100 **Table 1:** Species/families of birds and collection sites

	Collection Sites						
	Molete's Oja Oba Market	Onireke Bird's Market	Shasha Market	UI Zoological Garden	Research Animal Unit, UI	Free Range	Total
Species/Families							
Columbidae (e.g. Pigeon and Dove)	4	5	5	-	9	5	28
Psattaculidae (e.g. Parrot, Parrakreet)	-	8	-	6	-	-	14
Anatidae (e.g. Mallards and Wild Geese)	4	4	10	10	-	-	28
Ardeidae (e.g. Egrets and Herons)	-	-	-	-	-	15	15
Ploceidae (e.g. Village Weaver)	-	-	-	-	-	7	7
Phansianidae (e.g. Guinea fowl and Francolin)	-	-	9	-	-	-	9
Accipitridae (e.g. Eagle, Hawk)	-	4		5	-	-	9

101



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103 **Figure 1:** Study area and collection sites within Ibadan metropolis. Source: Google Map

PEER REVIEW

RESULTS

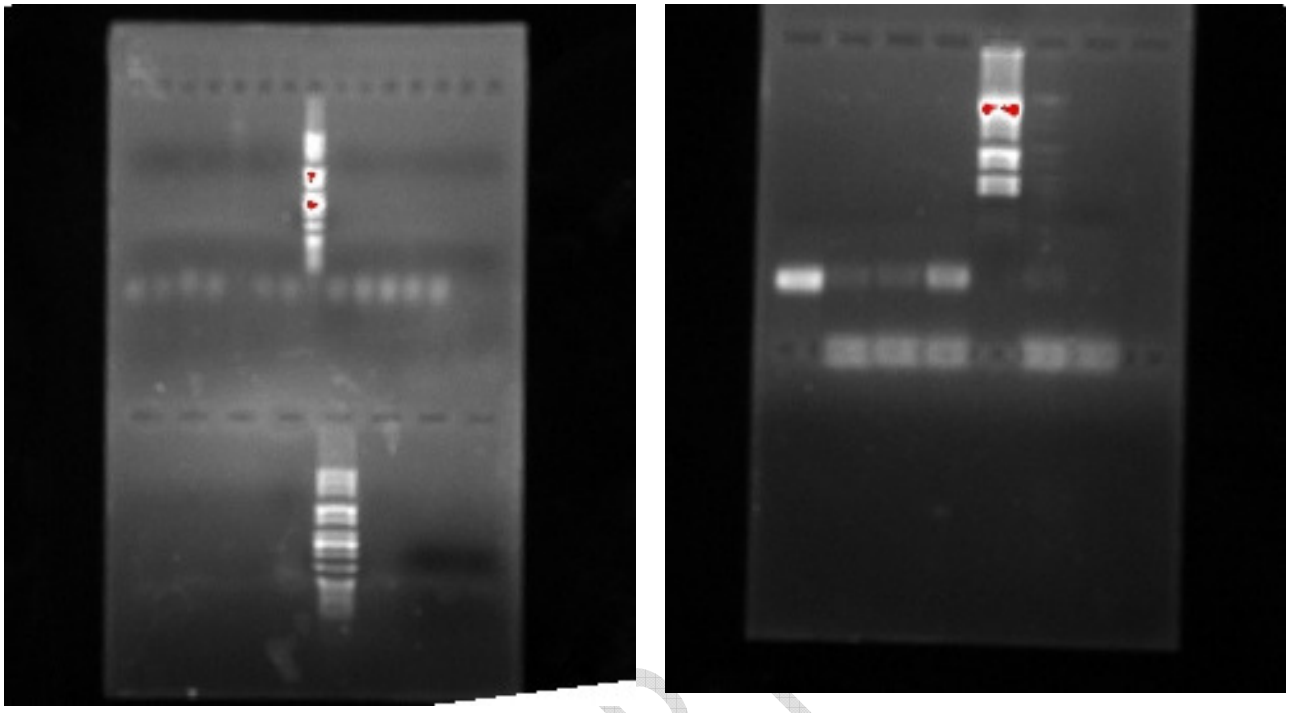

Figure 2: Gel picture showing no positive bands for the detection of West Nile virus

DISCUSSION AND CONCLUSION

Attempts to detect the targeted virus from the faecal matter of wild and peridomestic birds, by using species-specific West Nile virus primers failed. The inability to detect the West Nile virus indicates that faecal-oral route of transmission for the virus might not be possible in nature even though it has been achieved under controlled experimental conditions. Kipp *et al.* (2006) reported high titre value of viral particles in the faeces of the birds inoculated with approximately 4000 PFU. This large amount of inocula is likely not achievable in nature where the mode of transmission is usually through mosquito bites. Hence, the inability to detect the targeted virus may be attributed to absence or low level of viral particles in the samples.

Also, Lanciotti *et al.* (1992) identified one of the problems affecting virus isolation to the small amount of viable virus in the inocula which can make isolation take days to weeks. Reisen *et al.*, (2005) also corroborated this report that low rates of transmission or absence of the targeted viruses among the wild birds might be responsible for the inability to detect their presence during analysis. Weaver and Reisen (2010) reported that arboviruses frequently persist at low or even tenuous maintenance levels until some change in single or multiple factors facilitates rapid and widespread amplification. The implicated relevant factors that could contribute to this include circumglobal changes in climate and anthropogenic

UNDER PEER

factors, epidemiology, and viral genetics (Weaver and Reisen, 2010). Consequently, there may be need for improved assays which are sufficiently sensitive and specific enough for clinical and epidemiological purpose.

Conclusively, the virus was not detected in any of the birds screened. The absence of the virus was believed not to be as a result of procedural error. Birds in the locations stated above were not habouring the virus. However, while the virus may remain undetected in these birds, changes in the aforementioned factors that could facilitate their widespread amplification such as circumglobal changes in climate and anthropogenic factors, epidemiology, and viral genetics should be monitored. Continuous and active surveillance are needed to be able to detect their incidence whenever they occur in this region of the country and other regions as well.

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UNDER PEER REVIEW

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