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Attempted Detection of West Nile Virus from Wild and Peridomestic Birds within Ibadan Metropolis in Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author OSF designed the study and wrote the first draft of the manuscript. Authors OSF and DOF wrote the protocol and managed the analyses. Author TAA identified the birds and in collection of samples. All authors read and approved the final manuscript.

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ABSTRACT

Wild and peridomestic birds have been implicated in the transmission of West Nile virus (WNV), acting either as reservoirs or vectors. A team of researchers reported detection of West Nile virus (WNV) in the faeces of infected wild birds after being experimentally infected with the virus subcutaneously. This necessitated the need for this study, which is aimed at investigating 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, one hundred and ten (110) wild and peridomestic birds were screened for the presence of WNV using rtPCR method. 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, concluding that birds in those locations were not habouring the virus. We recommend continuous and active surveillance for the virus since change in some particular factors can aid its widespread transmission.

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1. 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 [1]. Birds can acquire or transmit viral infections via vertical or horizontal modes of transmission [2]. 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 [2,3]. Arboviruses are known to employ mosquito-bird interaction in their transmission cycles [2,4], and West Nile virus (WNV) is one of the most known arboviruses.

WNV 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 the Center for Food Security and Public Health [5], 326 birds have been associated with the virus, either by isolation or detection of its neutralizing 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 [6,7,8] while the viruses are being transmitted by multiple *Culex* species of mosquito [5,9,10]. 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 (SLEV) [11,12], acting as reservoirs and sometimes exhibiting pathological symptoms of the infection too. WVN has also been isolated from pigeons [5,13,14, 15], and persistent antibodies have been found in doves and pigeons in a set of studies [16,17]. Other implicated birds include ducks, geese and mallards [9,5] and, hawks and eagles [5,18].

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 [19]. In the review carried out by Kuno and Chang [18], 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. It was also 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 [20]. These reports indicate that faecal droppings of infected birds, both symptomatic and asymptomatic, are potential sources of infection for viruses that are 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 live in close proximity to human populations, hence increasing the chances of transmission of these viruses [20,21]. While it has been reported that arboviruses can be transmitted through ingestion of substances contaminated by faeces of infected hosts [2,22], it was demonstrated by Kipps et al. by isolating and detecting WNV in the faeces of American crows and fish crows which had been experimentally infected with the virus through subcutaneous inoculation [23]. 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 yet 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.

2. MATERIALS AND METHODS

2.1 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 Fig. 1. Domestic birds that were reared and sold at the population sites were excluded. Also, suspected birds that were not within Ibadan city were excluded.

Table 1. Species/families of birds and collection sites

Species/Families	Collection sites						
	Molete's Oja Oba market	Onireke bird's market	Shasha market	UI zoological garden	Research animal unit, UI	Free range	Total
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

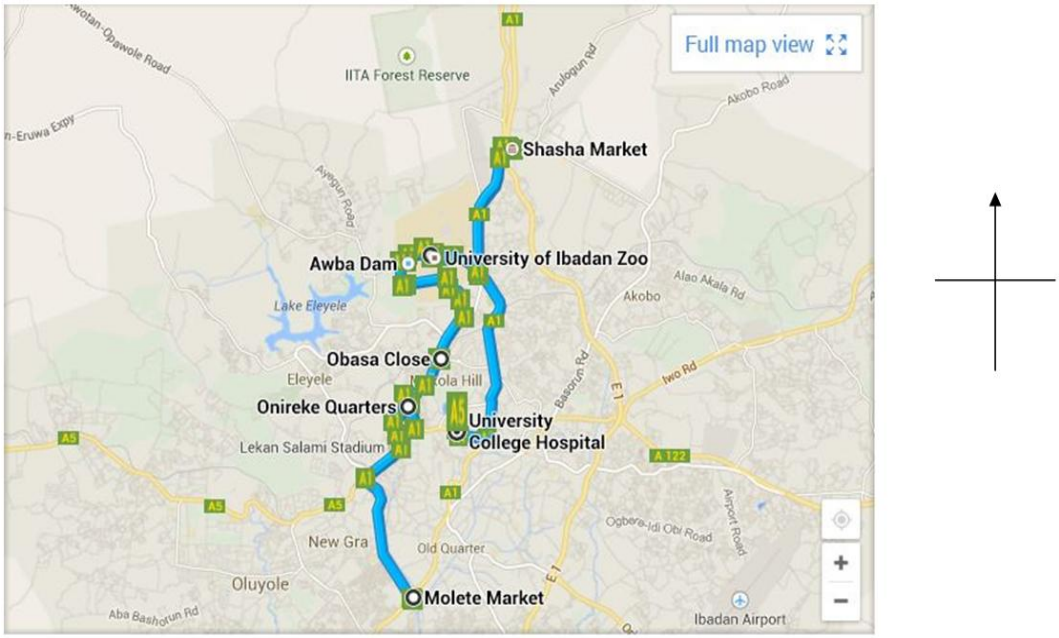

Fig. 1. Study area and collection sites within Ibadan metropolis
Source: Google Map

2.2 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°C until analyses.

2.3 Detection of Virus

Detection of suspected virus was done using rtPCR method. For rtPCR analysis, RNA was extracted from 140 ml of PBS-diluted faecal supernatant using viral RNA extraction kit (Jena Bioscience) according to the manufacturer's recommended procedure, and eluted with 60 ml sterile water. cDNA Synthesis and PCR amplification was carried out under conditions described by Bronzoni et al. [24] with modifications. 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°C for 10 min, followed by a further incubation at 50°C for 30-60 mins. The mixture was heated to 70°C for 10min to inactivate the reverse transcriptase. 2 units of DNase-free RNase was also added and incubated at 37°C for 20 min to remove RNA. The cDNA synthesized was now used as template to synthesize the second-strand using PCR and stored at -20°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. [25]. 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 follows: Taq Activation (94°C for 3mins); Template Denaturation (94°C for 30 secs); Annealing (50°C for 30secs); Template Elongation (68°C for 30 secs); Final Elongation (72°C for 7 mins). The expected amplicons sizes for first round and second round (nested) PCR are 464bp and 278bp respectively. Amplicons were analyzed using 3% agarose gel electrophoresis followed by ethidium bromide staining and UV visualization.

3. RESULTS

Attempts to detect the suspected virus from the faecal matter of wild and peridomestic birds, using the species-specific WNV primers given above, failed.

The DNA ladder used was graduated from 100bp to 1000bp. As shown in the gel pictures (Fig. 2), the targeted amplified regions of the virus were not detected in any of the samples, as the ladder regions around 464bp and 278bp showed no visible bands. The sample lanes shown were randomly selected among the 110 sample-specimens to highlight the lack of visible band in the analysis. (Please note that the red colouration seen in the gel picture has no bearing on the result in any way; it was as a result of the camera used to take the picture).

We did not detect WNV in any of the sample-specimen.

4. DISCUSSION

Attempts to detect the targeted virus from the faecal matter of wild and peridomestic birds, by using species-specific WNV primers failed. The inability to detect the West Nile virus indicates that faecal-oral route of transmission for the virus is not possible in nature even though it has been achieved under controlled-experimental conditions. Kipp et al. reported high-titre value of viral particles in the faeces of the birds inoculated with at approximately 4000 PFU [23]. This large amount of inocula in fecal-oral transmission is likely not achievable in nature where the mode of transmission is usually through mosquito-bites. Hence, the inability to detect the targeted virus in our study may be attributed to absence of or low-level of viral-particles in the samples.

Also, Lanciotti et al. identified one of the problems affecting virus-isolation to be the small amount of viable-virus in the inocula which can make isolation only possible after days to weeks [26]. Reisen et al., also corroborated this report of our study that low rates of transmission or absence of the targeted viruses among the wild

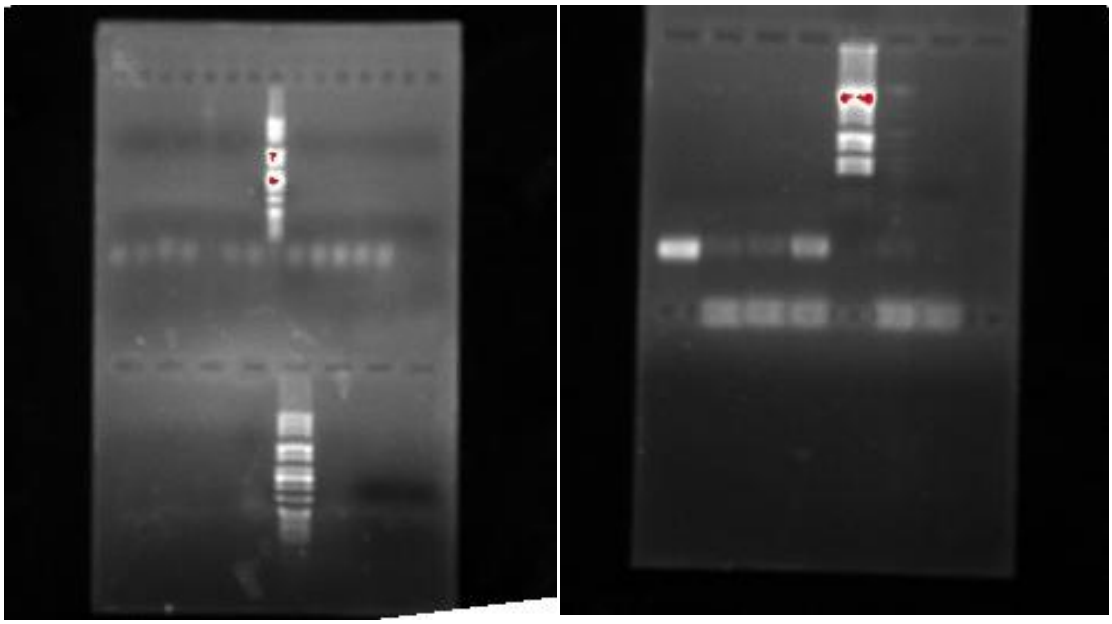

Fig. 2. Gel picture showing no positive bands for the detection of West Nile virus

birds might be responsible for the inability to detect their presence during analysis [8]. Weaver and Reisen 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 [3]. The implicated relevant factors that could contribute to this include circumglobal changes in climate and anthropogenic factors, epidemiology, and viral genetics [3]. Consequently, there is a need for improved assays which are sufficiently sensitive and specific enough for clinical and epidemiological purpose.

5. CONCLUSION

In conclusion, the virus was not detected in any of the birds screened. The absence of the virus is believed to be not as a result of procedural error. Birds in the locations stated above are not habouring the virus. However, while the virus may remain undetected in these birds, changes in the aforementioned factors that could facilitate its widespread amplification and transmission such as circumglobal changes in climate and anthropogenic factors, epidemiology, and viral genetics should be monitored. Continuous and active surveillance is to determine the incidence of virus-carriage in these birds in this region of Nigeria, and other regions as well.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

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The map in Fig. 1 was gotten from Goggle Maps.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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