Original Research Article

Molecular surveillance of common wild birds as potential hosts for selected zoonotic viruses within Ibadan, Nigeria

ABSTRACT

Wild birds have been known to be carriers and reservoir hosts for many zoonotic viruses. This necessitated this study which is aimed at detecting some zoonotic viruses in the faeces of selected wild birds within Ibadan, Southwest Nigeria. In carrying this out, faeces and cloacal swabs were collected from 110 birds from the avian families Columbidae, Psattaculidae, Anatidae, Ardeidae, Ploceidae, Phansianidae and Accipitridae across different locations across the city of Ibadan for a period of 12 months and screened for Alphaviruses, Flaviviruses, Rift Valley fever virus and Avian Paramyxovirus (Newcastle Disease Virus) using genus-specific and species-specific primers in a rtPCR method. The amplicons were subjected to 3 – 5% agarose gel electrophoresis for detection of the targeted amplified sequences. None of the targeted viral sequences was detected in the samples, showing the absence of the suspected viruses among the birds screened in this city. It is recommended that further surveillances of other species and genera of birds be continually carried out in order for early detection before potential outbreaks.

Key words: Zoonootic viruses, wild birds, Alphaviruses, Flaviviruses, Rift Valley fever virus, Avian Paramyxovirus, Southwest Nigeria.

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. Vertical transmission, also termed transovarial transmission, is usually from an infected parent to offspring, usually through the eggs (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). While arboviruses are mainly transmitted by employing a biological mode of transmission involving the virus replicating within an arthropod host before transmission (4), experiments have shown that some viruses could also be transmitted through ingesting of substances contaminated by faeces of infected hosts (2,5). Also, it has been reported that spread from bird to bird appears can occur as the result of ingestion of infective material such as faeces (6). These reports categorically indicated that faecal droppings of

infected birds, both symptomatic and asymptomatic, are potential sources of infection for viruses shed in birds' faeces.

Flaviviruses belong to the family Flaviridae of positive-strand RNA viruses and comprise more than 70 members including important human pathogens such as yellow fever virus, dengue virus, and West Nile virus. Flaviviruses can be divided into three groups based on the vector employed in spreading the viruses (2). These are the mosquito-borne group, the tick-borne encephalitis group, and the group with no recognised arthropod vectors. The mosquito-borne group can further be divided into two based on their neurotropism. Non-neurotropic viruses are associated with Aedes mosquitoes, primate reservoir hosts and haemorrhagic diseases in human. Examples include Dengue virus and Yellow fever virus. The neurotropic viruses are associated with *Culex* mosquitoes, bird reservoir hosts and encephalitic diseases in human or animal. Examples include Japanese encephalitis virus, West Nile virus, Usutu virus, St. Louis encephalitis virus and Murray Valley encephalitis virus (7)

Another family of viruses are the Alphaviruses, which are naturally maintained in birds. Birds are the usual vertebrate reservoirs of alphaviruses, although they are capable of infecting mammals, and have been isolated from amphibians and reptiles (2). Alphaviruses are members of the family Togaviridae. The group was initially referred to as group A arboviruses. Other genera under the family include Rubivirus and Pestivirus. There are over 30 alphaviruses within the genus Alphavirus and the viruses are transmitted to their vertebrate hosts by arthropods and have defined geographic distributions (7). According to many authors (7,8,9), a number of lineages or clades are present, including a clade of aquatic viruses, a clade of encephalitic viruses (Eastern equine encephalitis virus, Venezuelan equine encephalitis virus), the Sindbis clade (Aura virus and many strains of Sindbis virus), the Semliki Forest Virus clade and a clade of recombinant viruses (the Western equine encephalitis virus lineage).

Newcastle Disease virus (NDV) also known as avian paramyxovirus type 1 (APMV-1), is the causative agent of Newcastle disease in various avian populations and usually result into high mortality(2). It is naturally maintained in pigeons, but can also be found in 250 species of birds in 27 orders, which can either be symptomatic or asymptomatic (10). The virus has reportedly caused infections ranging from mild, self-limiting influenza-like disease with fever, headache and malaise to serious opportunistic infections in immunosuppresed individuals. Wild birds, especially waterfowls such as geese, ducks, egrets, herons and mallards have been reported to carry the virus asymptomatically and serving as reservoir for it in the process (6). The virus can be transmitted in faeces (ingestion) and respiratory droplets (inhalation), especially through aerosols. Velogenic strains of APMV-1 have been documented to cause conjunctivivtis in humans,

especially when exposed to large quantity of the virus, and it is said to mostly occur among laboratory workers and vaccination crews (10).

Rift Valley fever virus (RVFV) is not an arbovirus, but nonetheless a zoonotic one. The reported incidents of faecal-oral transmission of arboviruses and the possible transmission of the virus from its common domestic hosts to avian hosts necessitated the inclusion of RVFV into this study. RVFV is a member of the Bunyaviridae family of viruses that are transmitted by varieties of arthropods such as mosquitoes, sandflies, ticks and midges (7). According to many sources, (3, 11), the virus is now endemic in sub-Saharan Africa, having caused substantial outbreaks in countries like Kenya, Egypt, Somalia, Tanzania, South Africa and Zimbabwe with relatively large mortality rate. Although the most common vertebrate hosts for RVFV are domesticated animals such as sheep, cattle and goats (11), the commonest mosquito species associated with the virus is *Aedes,* which is also known to feed on wild, domesticated and peridomesticated birds (3). This necessitated the need to investigate birds as a potential carrier of the virus.

This study aims to investigate the potential of some wild birds in Ibadan, Nigeria as reservoir hosts and carriers in the transmission of the above-stated zoonotic viruses through their faeces.

MATERIALS AND METHODS

Study Population and Sites: A total of 110 specimens were collected. Cloacal swabs (n = 60) and faeces (n = 50) of identified wild birds from the families Columbidae, Psattaculidae, Anatidae, Ardeidae, Ploceidae, Phansianidae and Accipitridae were collected as presented in Table 1. The birds were selected according to their availability, and faecal/cloacal samples were collected across many locations within Ibadan metropolis. Sample size was randomly chosen based on the availability of the birds under study.

Table 1: Families of Birds, Type Birds and Specimen Number

Families of Birds	Type Birds	Number of Specimen
Columbidae	Pigeon, Dove	28
Psattaculidae	Love birds, Parrot, Parrakreet	14
Anatidae	Mallards, Wild Geese	28
Ardeidae	Egrets, Herons	15
Ploceidae	Village Weaver	7
Phansianidae	Guinea fowl, Francolin	9
Accipitridae	Eagle, Hawk, Lizard Buzzard	9

Sample Collection: Swabs of fresh faeces were taken from birds from free ranges (Ardeidae, Columbidae and Ploceidae), from those whose anuses were not wide enough or whose owners refused cloacal swabs (Psattaculidae), and from potentially dangerous birds (Anatidae and Accipitridae). Cloacal swabs were collected from the anus/cloacae of large birds and less dangerous birds (Phansianidae and Columbidae) .Samples collected were transported in transport medium to the laboratory, where they were stored at -20^{0}C until analyses.

Detection of viruses: Detection of suspected viruses 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: Reverse transcription was carried out using 1ml RNA, 0.2µl of each primer, 4µl RT Buffer (SCRIPT), 1µl dNTP mix, 1µl DTT stock solution, 1µl RNase Inhibitor, 0.5µl Reverse Transcriptase (SCRIPT) and RNase-free water, added up to make up a total volume of 20µl. The Reaction Mix was incubated at 50^{0}C for 10 min, followed by a further incubation at 50^{0}C for 30-60 min. The mixture was heated to 70^{0}C for 10min to inactivate the reverse transcriptase. 2 units of DNase-free RNase was also added and incubated at 37^{0}C for 20min to remove RNA. The cDNA synthesized was now used as template to synthesize the second-strand using polymerase chain reaction and stored at -20^{0}C. For amplification, each PCR reaction contained 2µl cDNA template, 3µl each primer, 2.5µl Taq Mix and 2.0µl Nuclease-free water, in a total volume of 12.5µl. The primers used in amplifying specific regions of the viral genomes of the target viruses are presented in **Table 2**. Thermocycling conditions using a 9700 model thermocycler (Applied Biosystems) were varied for each viral cDNA amplified as presented in **Table 3**. Product was analyzed using 3% agarose gel electrophoresis followed by ethidium bromide staining and UV visualization.

Primers	Sequences of Primers (5' – 3')	Amplified regions	Amplicon size (bp)
Alphavirus[1]			
M2W	YAGAGCDTTTTCGCAYSTRGCHW	NS1	434
cM3W	ACATRAANKGNGTNGTRTCRA ANCCDAYCC		
M2W2	TGYCCNVTGMDNWSYVCNGARGAYCC		
Flavivirus[2]			
FU1	TACCACATGATGGGAAAGAGAGAGAA	NS5	310
CFD2	GTGTCCCAGCCGGCGGTGTCATCAGC		
Avian Paramyxovirus[3]			
P1F	TTGATGGCAGGCCTCTTGC	F protein	362
P2R	GGAGGATGTTGGCAGCATT		
Rift Valley Fever[4]			
NSca	CCTTAACCTCTAATCAAC	NSs	600
NSng	TATCATGGATTACTTTCC		

Table 2: Primers used for synthesis and amplification of cDNA

[1] Bronzoni *et al*., (2005); [2] Bronzoni *et al*., (2005); [3]Oberdorfer and Werner (1998); [4]Sall *et al.,* (2002)

Table 3: Thermocycling Conditions for Amplification of cDNA Templates

Thermocycling Conditions	Temperature	Time
Alphavirus (First round)		
Taq Activation	94^{0}C	3mins
Template Denaturation	94^{0}C	30secs
Annealing	53^{0}C	1 mins
Template Elongation	72^{0}C	2 mins
Final Elongation	72^{0}C	10 mins
Number of cycle: 35		
Alphavirus (Second round)		
Taq Activation	94^{0}C	2mins
Template Denaturation	94^{0}C	30secs
Annealing	55^{0}C	30secs
Template Elongation	72^{0}C	30secs
Final Elongation	72^{0}C	10mins
Number of cycle: 45		
Flavivirus		
Taq Activation	94^{0}C	3mins
Template Denaturation	94^{0}C	30secs
Annealing	53^{0}C	30secs
Template Elongation	68^{0}C	30secs
Final Elongation	72^{0}C	7mins
Number of cycle : 50		
Rift Valley fever virus		
Taq Activation	94^{0}C	3mins
Template Denaturation	94^{0}C	30secs
Annealing	45^{0}C	30secs
Template Elongation	68^{0}C	1min
Final Elongation	68^{0}C	7mins
Number of cycle: 40		
Avian Paramyxovirus		
Taq Activation	94^{0}C	3mins
Template Denaturation	94^{0}C	30secs
Annealing	53^{0}C	30secs
Template Elongation	72^{0}C	30secs
Final Elongation	72^{0}C	7mins
Number of cycle: 55		

RESULTS

Attempts to detect the suspected viruses from the faecal matter of the selected wild birds, by using genus-specific alphavirus primers (for alphaviruses) genus-specific flavivirus primers (for flaviviruses), species-specific avian paramyxovirus primers and species-specific Rift Valley fever virus primers failed.

DISCUSSION

The inability to detect any of the targeted viruses may be attributed to absence or low level of viral particles in the samples. One of the problems affecting virus isolation has been attributed to the small amount of viable virus in the inocula which can make isolation take days to weeks (12). Consequently, this has created a need for improved assays which are sufficiently sensitive and specific enough for clinical and epidemiological purpose even in the absence of viable virus.

The failure to detect some of the viruses despite using RT-PCR might be due to the susceptibility to adverse conditions associated with enveloped viruses which included alphaviruses and the flaviviruses (2). The low rates of transmission or absence of the targeted viruses among the wild birds whose faeces and cloacal swabs were screened also corroborate the reports and reviews carried out by many authors (13,14,15).

It has been 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 (derived from human activities) factors, epidemiology, and viral genetics (3).

In the study carried out in detecting avian paramyxovirus from wild and captive birds (15), the authors suggested that the low rate of isolation and detection of the virus from wild birds is an indication that wild birds may not be the carrier of the virulent strain of the virus, hence may not play any part in the maintenance of the virus in domestic avian population. However, as pointed out in the same report, highly virulent strains could evolve from viruses of low virulence by mutation; therefore there is need for constant surveillance and comparison of isolated viruses to known ones.

The assertions made in some studies (7,11) that the commonest hosts for RVFV are domesticated animals such sheep and goat instead of birds may be correct. Although many animals such as

domestic cattle, sheep and goat have been identified as the hosts of the virus (16), the reason for the inclusion of birds in this study is because of the possibility of mosquito transmission of the virus through bites. The inability to detect the virus from the faeces of these birds is an indication that birds are not playing a role as hosts for the virus in this location.

CONCLUSION

In conclusion, the suspected viruses were not detected in any of the birds screened. The absence of these viruses is believed not to be as a result of procedural error. Birds in the location stated above are not habouring the suspected viruses. However, continuous and active surveillance is recommended to determine the incidence of virus-carriage in these birds in this region of Nigeria, and other regions as well.

REFERENCES

1. Jacob Jacquie, Pescatore Tony and Cantor Austin. 2011. Avian diseases transmissible to humans. University of Kentucky, College of Agriculture.
2. Strauss, James and Strauss, Ellen (2008) Viruses and Human Diseases. Second Edition. Division of Biology, California Institute of Technology, Pasadena, California. Elsevier's Science & Technology.
3. Weaver S. C. and Reisen W. K. 2010. Present and future arboviral threats. *Antiviral Research* 85: 328–345
4. Weaver S.C., Kang W., Shirako Y., Rumenapf T., Strauss E.G., Strauss J.H. Recombinational history and molecular evolution of western equine encephalomyelitis complex alphaviruses. *J Virol* 1997, 71:613-623
5. Kipps A.M., Lehman J.A., Bowen R.A., Fox P.E., Stephens M.R., Klenk K., Komar N., Bunning M.L. West Nile Virus quantification in faeces of experimentally infected American and fish crows. *American Journal of Tropical Medicine and Hygiene.* 2006; 75(4):688–690.
6. Alexander, D. J. 2000. Newcastle disease and other avian paramyxoviruses. *Rev. sci. tech. Off. int. Epiz.*19 (2): 443-462
7. Zuckerman A. J., Banatvala J. E., Schoub B. D., Griffiths P. D. and Mortimer P. 2009. *Principles and Practice of Clinical Virology, Sixth Edition* John Wiley & Sons Ltd. ISBN: 978-0-470-51799-4.
8. Reisen W. K. (2003) Epidemiology of St. Louis encephalitis virus. Adv Virus Res 61: 139–183.
9. Pfeiffer Martin and Dobler Gerhard. Emergence of zoonotic arboviruses by animal trade and migration. *Parasites & Vectors* 2010, 3:35
10. The Centre for Food Security and Public Health (CFSPH). Newcastle Disease. 2005. Updated July 18, 2008
11. World Health Organisation. Information and facts about Rift Valley fever. Updated October 2016.

12. Lanciotti R.S., Calisher C.H., Gubller D.J., Chang G.J. and Vorndam A.V. 1992. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-olymerase chain reaction. *J Clin Microbiol* March; 30 (3): 545-51.

13. Miller, Patti J., Afonso Claudio L., Spackman Erica, Scott Melissa A., Pedersen Janice C., Senne Dennis A., Brown Justin D., Fuller Chad M., Uhart Marcela M., Karesh William B., Brown Ian H., Alexander Dennis J., and Swayne David E. Evidence for a New Avian Paramyxovirus Serotype 10 Detected in Rockhopper Penguins from the Falkland Islands *J. Virol.* 2010, 84(21):11496-11504.

14. Reisen, William K., Wheeler Sarah, Armijos M. Veronica, Fang Ying, Garcia Sandra, Kelley Kara, and Wright Stan. Role of Communally Nesting Ardeid Birds in the Epidemiology of West Nile Virus Revisited. *Vector-Borne and Zoonotic Diseases* 2009. 9 (3): 275 – 280.

15. Ibu, O.J., Okoye, J.O.A., Adulugba E.P., Chah K.F., Shoyinka, S.V.O., Salihu, E., A.A. and Chukwuedo, Baba, S.S. Prevalence of Newcastle Disease Viruses in Wild and Captive Birds in Central Nigeria. *Int. J. Poult. Sci.,* 2009 8 (6): 574-578.

16. Fischer Egil A.J., Boender Gert-Jan, Nodelijk Gonnie, de Koeijer Aline A. and van Roermund Herman J.W. The transmission potential of Rift Valley fever virus among livestock in the Netherlands: a modelling study. *Veterinary Research* 2013, 44:58.

17. Bronzoni, Roberta Vieira de Morais; Fla ´via Graciela Baleotti, Rita Maria Ribeiro Nogueira, Ma ´rcio Nunes, and Luiz Tadeu Moraes Figueiredo (2005) Duplex Reverse Transcription-PCR Followed by Nested PCR Assays for Detection and Identification of Brazilian Alphaviruses and Flaviviruses *Journal Of Clinical Microbiology*, 2005 43 (2): 696–702

18. Johnson, Donna J., Ostlund, Eileen N., Pedersen, Douglas D., and Schmitt, Beverly J. (2001) Detection of North American West Nile Virus in Animal Tissue by a Reverse Transcription-Nested Polymerase Chain Reaction Assay. *Emerging Infectious Diseases* Vol. 7, No. 4, July–August 2001.

19. Oberdorfer, Angela and Werner, Ortrud (1998) Newcastle disease virus: Detection and characterization by PCR of recent German isolates differing in pathogenicity. *Avian Pathology*, 27:3s 237-243, DOI: 10.1080/03079459808419330

20. Sall A.A., Macondo E.A., Sène O.K., Diagne M., Sylla R., Mondo M., Girault L., Marrama L., Spiegel A., Diallo M., Bouloy M., and Mathiot C. Use of Reverse Transcriptase PCR in Early Diagnosis of Rift Valley Fever *Clin Diagn Lab Immunol.* May 2002; 9(3): 713–715.