Asian Journal of Research in Infectious Diseases

2(3): 1-8, 2019; Article no.AJRID.48904

Molecular Surveillance of Common Peridomestic Wild Birds as Potential Hosts for Selected Zoonotic Viruses within Ibadan, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author OSF designed the study, wrote the protocol, managed the analyses and wrote the first draft of the manuscript. Author TAA located and identified the birds and handled sample collections. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRID/2019/v2i330102
Editor(s):
(1) Dr. Shahzad Shaukat, Department of Virology, National Institute of Health,Islamabad, Pakistan.
(2) Dr. Win Myint Oo, Associate Professor, Faculty of Medicine, Sibu Clinical Campus, SEGi University, Malaysia.
Reviewers:
(1) Muhammad Salihu Abdallah, Yobe State University, Damaturu, Nigeria.
(2) Wafaa Abd El-Ghany Abd El-Ghany, Cairo University, Egypt.
(3) Ronald Iorio, University of Massachusetts Medical School, USA.
Complete Peer review History: http://www.sdiarticle3.com/review-history/48904

Original Research Article

Received 12 March 2019
Accepted 24 May 2019
Published 30 May 2019

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 peridomestic wild birds within Ibadan, Southwest Nigeria. Peridomestic wild birds are defined for this study as wild birds that live close to, or are kept close to, human habitations, either for commercial purposes, entertainment purposes or as pets. 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

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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.

Keywords: Zoonootic viruses; peridomestic wild birds; alphaviruses; flaviviruses; rift valley fever virus; avian paramyxovirus; Southwest Nigeria.

1. INTRODUCTION

Wild 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 peridomestic wild birds in Ibadan, Nigeria as reservoir hosts and carriers in the transmission of the above-stated zoonotic viruses through their faeces.

2. MATERIALS AND METHODS

2.1 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.

2.2 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 were collected from free-living birds by picking fresh faeces below their roosting sites, which mostly trees or buildings. Large pieces of cardboards and/or clothing were spread under these trees and buildings, and the distinct pieces of faeces that were passed on them were picked/swabbed. All collected samples collected were transported in transport medium to the laboratory, where they were stored at -20°C until analyses.

2.3 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.

2.4 cDNA Synthesis and PCR Amplification

Reverse transcription was carried out using 1ml RNA, 0.2 µl of each primer, 4 µl 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 50^0C for 10 min, followed by a further incubation at 50^0C for 30-60 min. The mixture was heated to 70^0C for 10 min to inactivate the reverse transcriptase. 2 units of DNase-free RNase was also added and incubated at 37^0C 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 -20^0C. For amplification, each PCR reaction contained 2µl 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 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.

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

Table 2. Primers used for synthesis and amplification of cDNA

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		

[1]Bronzoni et al. [17]; [2]Bronzoni et al. [18]; [3]Oberdorfer and Werner [19]; [4]Sall et al. [20]

3. RESULTS

Attempts to detect the virus families of Alphavirus and Flavivirus from the faecal matter of the selected peridomestic wild birds using the cited genus-specific alphavirus primers (for alphaviruses) and genus-specific flavivirus primers (for flaviviruses) respectively failed. Also, attempts to detect avian paramyxovirus (Newcastle disease virus) and Rift Valley fever virus using species-specific avian paramyxovirus primers and species-specific Rift Valley fever virus primers respectfully also failed.

The DNA ladder used was graduated from 100bp to 1000bp. The targeted amplified regions of the suspected viruses were not detected in any of the samples, as the ladder regions around the expected amplicons sizes (434bp, 310bp, 362bp and 600bp for Alphavirus, Flavivirus, Avian Paramyxovirus and Rift Valley fever virus respectively) showed no visible bands. The sample lanes shown were randomly selected among the 110 sample-specimens to highlight the lack of visible bands of the expected sizes in the analysis. The reasons for these results are given in the discussion section. (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).

4. 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

Table 3. Thermocycling conditions for amplification of cDNA templates

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

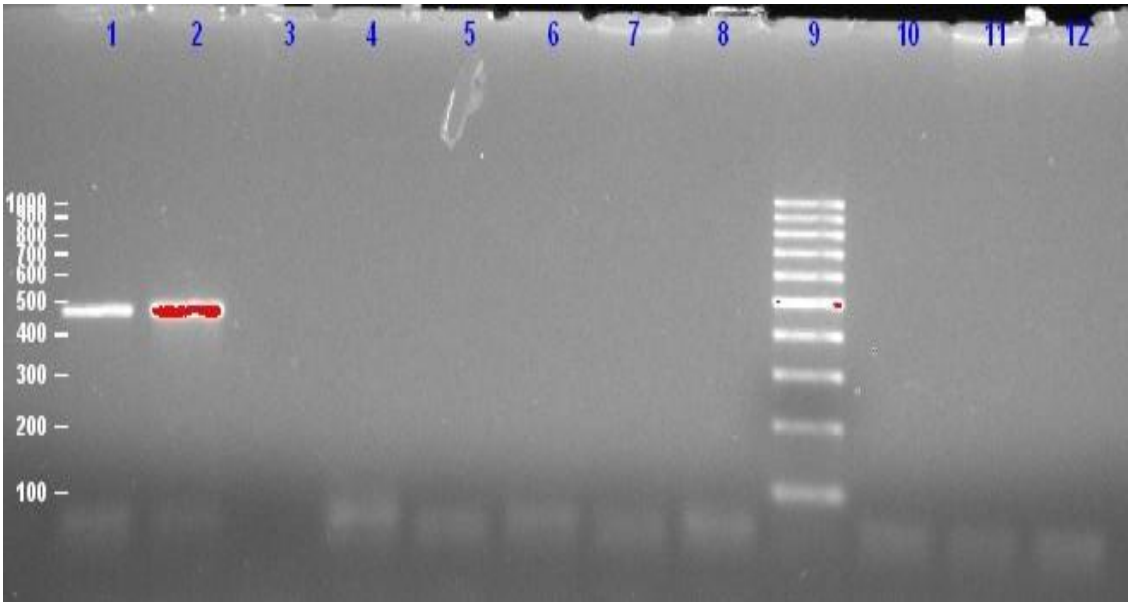

Fig. 1. Gel picture showing no positive bands of the expected amplicons sizes for the suspected viruses

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.

A general reason for the lack of detection of the suspected viruses in these birds has to do with their peridomesticity. All the wild birds whose samples were collected lived around human habitations, and were not known or shown to have come in contact with wild birds that lived in the jungle. One of the identified factors that aid transmission of infectious agents among birds is increased interactions with wild, jungle birds, especially through migrations [2,5,9]. Studies have also shown that viruses could be transmitted from birds to birds by feeding an infected host/carrier of the virus or virus-contaminated foods or drinks [3,5,6].

Most of the wild birds whose samples were collected were captive birds that were raised and/or kept in zoos, markets and research facilities where the feedings were controlled and there are lesser chances of migration or interaction with other birds. The birds exempted from this status were the Egrets/Herons, some pigeons/doves and the village weavers. However, it should be noted that all the birds, including the latter, were all living close to human habitations, and not in the jungle where they would be able to interact with other wild birds. These reasons contributed hugely to the lack of detection of the targeted viruses.

5. 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 because of the reasons stated above. 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 since migratory habits can change and increased interactions with other wild birds, either through roosting or feeding.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/48904