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The Role of Bridge Species in the Transmission of Avian Influenza and Newcastle Disease Viruses in Jos, Nigeria

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Abstract
Avian influenza has increasingly received attention over the years, primarily because of its ability to cause serious threat to the welfare of wild bird population, agriculture and human health. This study sort to understand the role bridge species play in the transmission of Avian Influenza and Newcastle Disease. Four hundred and fifty three birds comprising of 58 species and 21 families were caught using mist netsin Jos, Nigeria. One hundred and forty nine cloacal and tracheal samples were analysed molecularly to test for presence of Avian Influenza (AI) and Newcastle Disease (ND) viruses. All samples tested negative for AI. Fifty three per cent (53%) of the samples analysed showed positive results for the ND viruses. There was no significant difference between the number infected and the number uninfected with ND viruses (χ^2 = 0.64, df = 1, p = 0.4233). Grey-headed Sparrow (Passer griseus), Red-checked Cordon Bleu (Uraeginthusbengalus), Village Weaver (Ploceuscucullatus), Red-billed Firefinch (Lagonostictasenegala) and Northern Red Bishop (Euplectesfranciscanus) were the most common species caught. Grey-headed Sparrow showed a 20% rate of infection, but there was no significance in number infected and number uninfected within the species (χ^2= 3.5, df = 1, p = 0.0606). This study also presents 58 species of birds that serve as "bridge species" in the study area.The results showed that bridge species play a role in the transmission of viruses. However, poultry that are reared in bird-proof systems with no contact with bridge species is recommended as there will be no link to transmit viruses.

Keywords: Avian influenza, Bridge-species, Feral birds, Pathogenicity, Poultry farms

INTRODUCTION
Avian influenza (AI), a viral disease formally referred to as fowl plaque, has increasingly received attention over the years. This is not only because of its ability to cause serious threat to the welfare of wild bird population, but also its effects on agriculture and human health (Clark and Hall, 2006). Most importantly, in recent years, there has been an alarming outbreak

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of the highly pathogenic avian influenza (HPAI) disease. This has resulted in high impact in terms of the number of birds involved and the costs of disease control(Dharmayanti *et al*., 2018). The sudden emergence and spread of the HPAI H5N1 virus in continents, is particularly of great concern (Capua and Alexander, 2006). Similarly, Newcastle Disease (ND), another disease of birds is regarded throughout the world as one of the two most important diseases of poultry and other birds, the other disease being highly pathogenic avian influenza (HPAI). The significance of ND is emphasized by the potential of great economic losses and constant dangers of introduction into disease free regions (Butt *et al*., 2018).

Some groups of wild birds that are not closely linked to wetland habitats but usually found in human disturbed habitats, have been shown to be fatally infected by HPAI (FAO, 2007). Among these are several species of songbirds or perching birds (Passeriformes) and the ubiquitous feral pigeons (*Columba livia*) of the Columbiformes order. These birds have a variety of habitat preferences, but also show a high tolerance for anthropogenic areas where they visit for food resources. Because of this close association with humans and subsequent contact with domestic birds, especially at open poultry farms, these birds act as "bridge species" (resident wild birds that come around domestic areas and still relate with their wild congeners).This enables them to serve as an important link in the transmission of AI and ND viruses between wild birds in natural habitats and domestic birds. Current studies on the ecology of influenza viruses suggest that all known subtypes of feral birds may be harbouring large pools of influenza A viruses. The outbreaks of highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI) in domestic poultry may be as a result of introduction from feral birds (Manu, 2009).

Poultry that are reared in such a way that allows contact with bridge species creates a link for the transmission of the influenza and Newcastle disease viruses, but less likely in poultry reared in bird-proof concealment. This knowledge will allow strategies to be developed to prevent introduction into poultry. However, many countries still engage in methods that encourage contacts with wild birds, such as surface storage of drinking water, rearing mixed species on same farm, exposure of food stores and construction of artificial dams that attract waterfowls.

Understanding the importance of AI and ND requires a clear knowledge of the structure of the agent and mechanism of disease within the context of the host range and ecological factors affecting transmission (Clark and Hall, 2006). The aim of this study was to understand the role bridge species play in the transmission of AI and NDV.

MATERIALS AND METHODS

Study area

This research was carried out in Jos, Nigeria, located on Latitude 08^{o}24′ north and Longitude 08^{o}32′ and 10^{o}38′ east. It is situated on an altitude of 1222 m.a.s.l. The first outbreak of highly pathogenic avian influenza virus in Nigeria was in Kaduna, in early January 2006. The outbreak had spread to additional fifteen states of the country including Plateau, where Jos is located. The outbreak started in commercial layers but later spread to other domestic and wild birds (Kumbish *et al.,* 2006a; Kumbish *et al.,* 2006b).

Study area

Ten poultry farms in Jos were visited in this study. Five sites each were selected around Jos South and North with particular attention to size and proximity to human habitation. Poultry

farms with over two hundred birds and at least 100m away from human habitation were considered.

Research design

Sampling was carried out for two days at each of the ten poultry farms. On each farm, three 20m mist nets were erected between 50m and 200m away from the farm (this distances were determined by the location of the farms around human habitation) to trap wild birds. The nets were visited at 20 minute intervals for extraction of birds. Mist netting was carried out in the mornings between the hours of 7:00amand 10:00am. Birds extracted were identified and sorted into species with the aid of a guide to Birds of Western Africa (Borrow & Demey, 2013). Birds trapped were ringed, aged, sexed and their biometric measurements taken. Cloacal and trachea samples were collected with the aid of sterilized swabs. Cryovials containing viral transport medium (VTM) were used to store and transport samples to the laboratory on ice park. The reverse transcriptase polymerase chain reaction (RT-PCR) was used to test for the presence of AI and ND viruses.

Molecular technique

One hundred and forty nine (149) samples of the four hundred and fifty three (453) total cloacal and tracheal samples collected from the field were analysed. The samples analysed were randomly selected and in consideration of the cost of analyses. This analysis was carried out in the molecular laboratory of the University of Ibadan, Nigeria, to check for the presence of the viruses.

RNA extraction

Ribonucleic acids (RNAs) of the samples collected were extracted using Qiamp Viral RNA minikit and eluted into 50 µl nuclease free H_2O. Extraction was carried out in an extraction hood and the necessary precautions to prevent contamination of samples were taken. The AVL (lysis buffer) was prepared by adding 1ml of AVL buffer to a carrier RNA red tube and mixed properly to re-suspend the powder. The solution was then transferred into the AVL bottle and the red tube was then rinsed with 1ml of AVL buffer and again transferred into the AVL bottle and the carrier RNA was added on the bottle lid. Aliquot of the solution (AVL + carrier) were made into 1.5ml eppendorf: 560µl/tube and labelled AVL, this was then stored at 4°C. Ethanol was added to the buffers AW1 and AW2, all in preparation for the extraction. The samples were decontaminated by placing on tissue paper and spraying with Virkon, wiped with tissue paper and labelled appropriately. The samples were equilibrated at room temperature and vortexed. The AVL buffer aliquots were then removed and re-dissolved (5min at 80°C with the heater block) and allowed to cool to room temperature before it was used. This was to dissolve any crystals that were formed from the freezing. 140µl of the samples were added each to 560µl of AVL and mixed properly by pulse vortexing for 15 seconds and incubated for 10 minutes at room temperature to lyse the virus. The mixture was spined to remove drops from the lids and 560µl of Ethanol was added and vortexed for 15 seconds. The mixture was spined and 630µl of the mixture was transferred into the labelled spin columns and spined in a centrifuge at 8000 rpm for 1 minute. The collection tubes were then discarded and replaced with new ones. The remaining 630µl of the mixture (140µl of sample + 560µl of AVL + 560µl of ethanol) were transferred into the same labelled spin columns and spined at 8000rpm for 1 minute and the collection tubes were again discarded and replaced with new ones. After this repeated process, 500µl of AW1 wash buffer was added to the spin column and then transferred to a centrifuge and spined at 8000rpm for 1 minute, and the collection tubes were changed. 500µl of AW2 wash buffer was then added to the spin columns, placed in the centrifuge again and spined at 13000rpm for 3 minutes and the tubes changed. The leblled spin columns were then spined for 1 minute at 13000rpm in a centrifuge

and collection tubes were discarded once more. Each labelled spin columns was then placed on a 1.5ml labelled eppendorf tube and to it, 60µl of elution buffer was added to the centre of each column and incubated at room temperature for 1 minute and then spined at 8000rpm for another 1 minute. The labelled spin columns were then discarded and the RNA was stored at - 80°C.

Viral detection

The RNAs extracted from the samples were screened for the presence of genomic nucleic acid from types-A influenza viruses and Newcastle disease viruses by means of reverse transcription polymerase chain reaction (RT-PCR) optimized with respect to primer sets, enzymes, and concentration of reagents as well as cycling parameters (Spackman,2004).

Statistical analysis

The R-Statistical package (version 2.9.2, R Development Core Team, 2018) was used to analyse the dataat 95% confidence level. Chi-Square model was used to run the analysis to check for significant difference between species of infected individuals, and whether or not there was significant difference in the individuals within species that were infected and those that were not.

RESULTS AND DISCUSSION

Wild bird surveillance

Four hundred and fifty three birds (453) consisting of 58 species and 21 families were caught in mist nets. Out of the 58 bird species recorded, 5 species were the most common namely; Grey-headed Sparrow (*Passer griseus*), Red-checked Cordon Bleu (*Uraeginthusbengalus*), Village Weaver (*Ploceuscucullatus*), Red-billed Firefinch (*Lagonostictasenegala*) and Northern Red Bishop (*Euplectesfranciscanus*). Red-checked Cordon Bleu, Village Weaver and Red-billed Firefinch appeared in all sites except one, while Grey-headed Sparrow and Northern Red Bishop appeared in four and six sites respectively across the study sites. The 58 species of birds caught in this study represents some of the bridge species that are found around Jos. The species are classified generally into four feeding guilds (granivores, Insectivores, frugivores and nectivores). Most of the species caught were granivores.

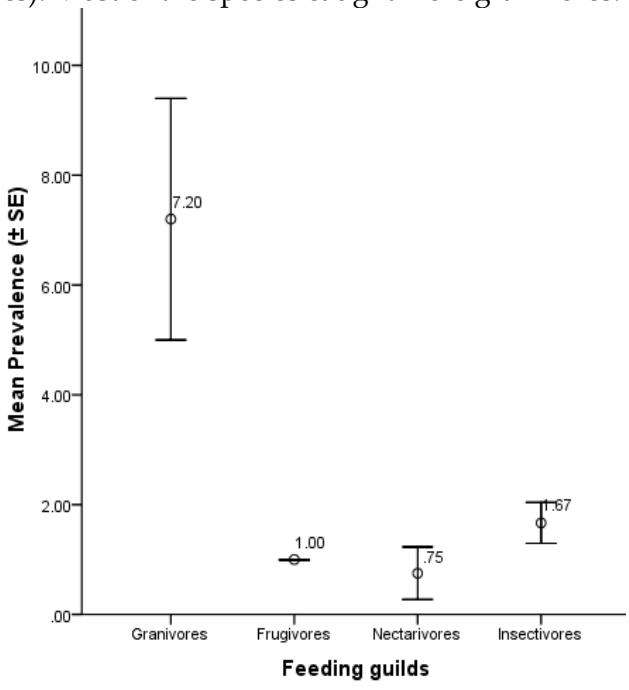

Figure 1: Mean prevalence of Newcastle disease between feeding guilds

Resident wild birds (bridge species) have been established from this study to visit active poultry farms particularly for food as they move about their daily activities, this has also been reported by Manu (2009) that wild birds are found in poultry farms where they visit for food. Most of the bird species caught were granivores, and this may be because poultry feeds constitutes mainly grains. Species from other feeding guilds like frugivores, insectivores and nectivores were also caught. This was mainly due to locations of some of the farms and its surrounding vegetation cover. Although vegetation parameters were not taken to determine the distribution patterns of the species caught, some of the poultry farms were located around flowing streams, while some others had farmlands surrounding them, which accounted for rare species (species that were caught once or twice). Such include Grey-headed Kingfisher (*Halcyon leucocephala*), Pygmy Kingfisher (*Ispidinapicta*), Yellow-billed Oxpecker (*Buphagusafricanus*) and the Cardinal Woodpecker (*Dendropicosfuscescens*).

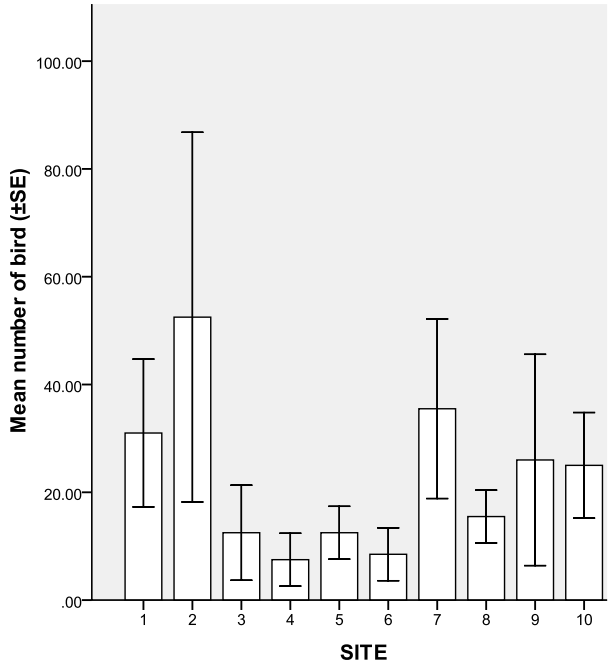

Figure 2: Mean number of bird species caught in each site

Molecular analyses

All samples tested negative for AI. Seventy nine (53%) samples tested positive for NDV viruses, while Seventy (47%) tested negative as seen in Table 1. There was no significant difference between the number of infected and uninfected birds (Chi-square: $\chi^2 = 0.64$, df = 1, $p = 0.4233$).

Table 1: NDV results of the total species analysed for each site

Site	Samples	Positive (NDV)	Negative (NDV)
1	23	12	11
2	36	24	12
3	9	4	5
4	7	5	2
5	6	2	4
6	5	2	3
7	31	13	18
8	5	2	3
9	11	6	5
10	16	9	7
Total	149	79	70

Grey-headed Sparrow (GRHSP) showed no significant difference within species (Table 2) when number infected were compared with number uninfected (Chi-square: $\chi^2 = 3.5$, df = 1, $p = 0.0606$). Grey-headed Sparrow had the highest number sampled and, although they were found in only four sites, an average of seven per sites will be seen if divided by the ten sites.

Table 2: Showing the NDV results of GRHSP in each site

Sites	Species	Positive (NDV)	Negative (NDV)
1	GRHSP	2	0
2	GRHSP	8	1
3	GRHSP	X	X
4	GRHSP	X	X
5	GRHSP	X	X
6	GRHSP	X	X
7	GRHSP	5	6
8	GRHSP	X	X
9	GRHSP	1	0
10	GRHSP	X	X
Total	23	16	7

Wild birds are a natural host to many viruses and so in most cases do not show symptoms to their infections (Ellis *et al*. 2004) but are capable of transmitting these viruses to other hosts like the domestic birds (FOA, 2005; Szeleczky *et al.* 2009).

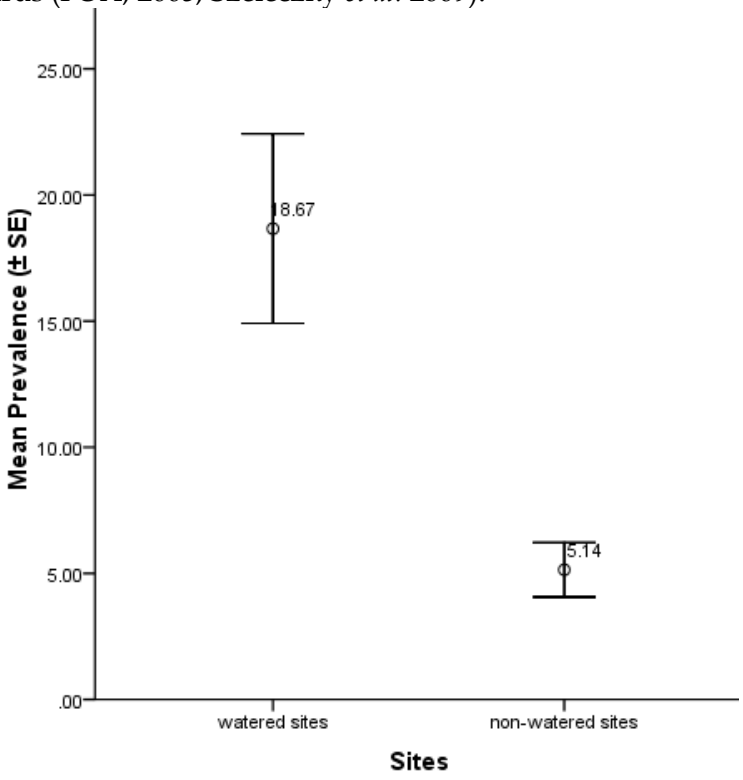

Figure 3: Mean prevalence of Newcastle disease between sites

The habitat type and/or geographical location of a place have been reported to influence the type and prevalence of the viruses (Sol *et al*., 2000; Freeman-Gallant *et al.* 2001; Wood *et al*., 2007). In this study, sites 1, 2 and 7 showed more positive cases of the ND viruses. These sites were located in habitats that had streams flowing nearby with many trees and considerable grassland, which together gave a heterogeneous pattern and so accounted for more diverse and higher numbers of species.

CONCLUSION

The importance of AI and ND viruses is not only in the fact that they cause diseases to birds and can lead to flock mortality rates of up to 100%, but also the economic impact that may ensue due to trading restrictions and embargos placed on regions and countries where such outbreaks occur. This study has shown an infection rate of 53% in NDV which is very high and is a risk to poultry farms around Jos. There might have been no positive cases of the AI viruses, but this does not necessarily mean that it is absent since it is also possible that infected birds could have died not long after infection with the deadly virus. Bridge species too have been identified and established that they carry ND viruses that are very important to livestock, and since there is a lot of contact with domestic birds and their wild congeners, there is a high possibility of the transmission and spread of ND viruses.

RECOMMENDATIONS

Considering the potential for the introduction of HPAI and ND viruses by wild birds, active surveillance should be put in place to provide an early warning system to allow for the improved protection of poultry and public health. To this effect, a working committee of wildlife biologists, veterinarians, virologists and public health experts should be formed by the Federal and State Governments, and saddled with the responsibility of providing guidelines for agencies and programs for wild bird surveillance. The Government should also strengthen the border protection, since one of the main risks of introduction of viruses is through illegal importation of poultry and poultry products, and through the illegal trade of wild and exotic birds.

REFERENCES

Borrow, N., Demey, R. (2013).*Birds of Western Africa*. London: Christopher Helm, pp 511.

Butt, S. L., Taylor, T. L., Volkening, J., Dimitrov, K. M., Williams-Coplin, D., Lahmers, K. K., . . . Stanton, J. B. (2018). Rapid and sensitive virulence prediction and identification of Newcastle disease virus genotypes using third-generation sequencing. *bioRxiv*, 349159.

Clark, L., Hall, J. (2006). Avian Influenza in Wild Birds: Status as Reserviors, and Risks to Humans and Agriculture. *Ornithological Monogragh*,**60**: 3-29.

Dharmayanti, N. L. P. I., Thor, S. W., Zanders, N., Hartawan, R., Ratnawati, A., Jang, Y., . . . ,Pudjiatmoko. (2018). Attenuation of highly pathogenic avian influenza A (H5N1) viruses in Indonesia following the reassortment and acquisition of genes from low pathogenicity avian influenza A virus progenitors. *Emerging microbes & infections*, **7**(1): 1-14.

Ellis, T. M., Bousfield, R. B., Bissett, L. A., Dyrting, K. C., Luk, G. S., Tsim, S. T., Sturm-Ramirez, K., Webster, R. G., Guan, Y., Malik Peiris, J. S. (2004). Investigations of outbreaks of highly pathogenic H5N1 avian influenza in water fowl and wild birds in Hong Kong in late 2002. *Avian Pathology*,**33**:492-505.

FAO (2007). Wild Birds and Avian Influenza: an introduction to applied field research and disease sampling techniques. Whitworth, D., Newman, S.H., Mundkur, T., and Harris, P. (eds). *FAO Animal production and Health Manual*, No. 5. Rome.

FAO Technical Task Force on Avian Influenza (2005). Potential risk of highly pathogenic avian influenza (HPAI) spreading through wild water bird migration. *FAO AIDE News* (Special Issue) **33**: 1–7.

Freeman-Gallant, C. R., O'Connor, K. D., Breuer, M. E. (2001). Sexual selection and the geography of *Plasmodium* infection in savanna sparrows (*Passerculussandwichensis*). *Oecologia*,**127**: 517-521.

Kumbish, P. R., Bello, M. K., Jambalang, A. R., Hussaini, B. A., Solomon, P., Ekong, P. S., Damina, M. S., Elisha, I. A., Chukwukere, S., Bako, B., Nanbol, D., Akanbi, B. O., Joannis, T. M., Ularamu, H. G., Shittu, A. I., Suleiman, L. K. (2006a). Bacterial and Parasitic

Agents Associated with Outbreaks of Highly Pathogenic Avian Influenza (H5N1) in Nigeria. *Vom Journal of Veterinary Science,***03**: 23-31.

Kumbish, P. R., Joannis, T. M., Jambalang, A. R., Damina, M. S., Hussaini, B. A., Akanbi, B. O., Oyetunde, I. L., Abdu, M. H., Danbirni, S., James, A., Yahaya, K., Bako, B., Nanbol, D., Elisha, I. A., Solomon, P., Ogunsan, E. A., Adulugba, A. P., Okewale, P. A. (2006b). Clinico-Pathological Features of Highly Pathogenic Avian Influenza (HPAI-H5N1) Outbreaks in Commercial Chickens in Nigeria. *Vom Journal of Veterinary Science,* **03**: 13-22.

Manu, S.A. (2009). Final report on the study of the role of migratory and resident wild birds in the spread and maintenance of the HPAI virus in Nigeria. A report submitted to FAO/ECTAD – NADIS NIGERIA Project, pp 14.

R Development Core Team. (2018). R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Sol, D., Jovani, R., Torres, J. (2000). Geographical variation in blood parasites in feral pigeons: the role of vectors. *Ecography,* **23**: 307-314.

Spackman, E. (2004). Development of a real time reverse-transcription PCR for detection of newcastle disease virus RNA in clinical samples. *Journal of Clinical Microbiology*. **42**: 329–338.

Szeleczky, Z., Dán, A., Ursu, K. (2009). Four different sub-lineages of highly pathogenic avian influenza H5N1 introduced in Hungary in 2006–2007. *Veterinary Microbiology,***139**: 24–33.

Wood M. J., Cosgrove, C. L., Wilkin, T. A. (2007). Within-population variation in prevalence and lineage distribution of avian malaria in blue tits, *Cyanistescaeruleus*. *Molecular Ecology,***16**: 3263-3273.