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https://dx.doi.org/10.4314/nvj.v39i1.1 **ORIGINAL ARTICLE**

Migratory Waterfowls from Europe as Potential Source of Highly Pathogenic Avian Influenza Infection to Nigeria Poultry

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SUMMARY

Highly pathogenic avian influenza H5N1 was detected for the first time in Nigeria and Africa in 2006. The source of the outbreak was not immediately clear, but scientific opinions favoured multiple introductions. The need to evaluate available epidemiological and virological data to demonstrate most probable source of introduction is important for focused control efforts. In this study, we described ten years (2006-2016) scientific observations available through ecology, epidemio-surveillance and molecular virology of avian influenza in Nigeria. Nigeria is geographically located in the tropical humid region with abundant wetlands presenting ideal ambience for birdlife. Ornithological evidence also showed that the country lies in the path of two major migratory flyways from Europe and Asia providing opportunities for co-mingling of different species and age of wild birds with resident birds. Genomic characterization including phylogenetic analysis revealed a high degree of homology among virus strains isolated in Nigeria and those detected in migratory birds from Europe suggesting common source epidemic. This study agreed with postulation that migratory wild birds from Europe may be responsible for the introduction of highly pathogenic avian influenza subtype H5N1 to Nigeria. Feral waterfowls around water bodies thereafter bridge transmission to domestic poultry holdings whereas local trade and movement of poultry and poultry products may further spread infection locally. In the light of this understanding, routine risk based sentinel surveillance for avian influenza in domestic waterfowls in ecological hotspots is recommended for early detection and response thereby saving economic losses and mitigating public health emergencies.

Key words: Avian influenza, Migratory waterfowls, Nigeria, Poultry.

INTRODUCTION

Nigeria lies within latitude 13^{o}2321.12; 4^{o}1019.06 north of the equator and longitude 2^{o}3423.20; 14^{o}4218.48 east of the Greenwich meridian (Milich and Weiss, 1997). The country is in the tropical humid zone. Annual rainfall average varies from 500mm in the north (Kano and Sokoto) to about 2200mm in the south (Lagos and Benin) and influenced by two major seasons - wet and dry (Adejuwon *et al.*, 1990; Nicholson, 2013). Although there are broad eco-climatic variations among ecological zones, Nigeria has mean annual minimum, mean and maximum temperatures (± standard deviations) of 21.4±3.5, 27.1±2.7 and 32.8±3.4°C, respectively, while the overall mean relative humidity is 62±24.8% (Eludoyin *et al*., 2013). The climate and ecosystem supports wide range flora and fauna making Nigeria a rich biodiversity hub with abundant wildlife and domestic animals within the West-Central Africa bioclimatic region (Norris *et al*., 2010). The Guinean forests of West Africa are recognized as a biodiversity hotspot supporting up to 25% of the African mammalian fauna, and displaying significant endemism across a range of animal and plant groups (Norris *et al*., 2010). Phenologies of the Nigeria biotypes across West Africa sub region influence migratory birds that tarry when they fly over the Sahelian region. The Climate across the vast ecological zone that stretches from the Atlantic seaboard of Western Europe to the Pacific coast of Siberia is strongly seasonal. Hence, many species of bird from this region after breeding move south to winter in Africa and South or Southeast Asia. These birds from Europe migrate to Africa along flyways across or round the Mediterranean (Cambridge Conservation Initiative, 2010). Waterfowls, waders, birds of prey and over 2 billion songbirds migrate from Europe to sub-Saharan Africa each year (Hahn *et al*., 2009).

Nigeria falls in the international migratory flyways viz: East-Africa-Asia flyway, Atlantic-America and Black Sea/Mediterranean flyway, some of which intersects in the country. During winter in Europe (corresponding to Harmattan cold in Nigeria), the dry and dusty Harmattan wind blows over the Sahara in a north – eastern direction with hot day temperature/low humidity and cool at night (Hamilton *et al*., 1945). The moderate cold during this period provides micro climatic conditions for habitation of many wild bird species from Europe that are involved in the trans-Saharan migration for favourable conditions (Saino *et al*., 2003). The migration of these birds to favourable climatic zones in the tropics at winter is often regulated by innate circadian clock genes that may play a role in regulating photoperiodic responses and timing of life cycle events such as breeding and feeding. These directly influence changes in habitats for warmer conditions and locations (Bazzi *et al*., 2016). Abundance of wetlands, rivers, natural lakes, floodplains and dug out dams in Nigeria also makes the country a suitable abode for wintering in the course of the north-south trans-Atlantic journey by many of these birds (Saino *et al*., 2003). These migratory birds make several trips over the years to and from areas with known cases of Highly Pathogenic Avian Influenza (HPAI) from Northeast Asia through Mongolia to countries in Europe including Croatia, Romania, Germany, Finland, Siberia, Kazakhstan and Russia (Gilbert *et al*., 2006). The spread of avian influenza through migratory birds from Asia, Europe to Africa is suspected. This has resulted in enormous economic impacts on the poultry industry and also presents an important source of transmission to human thereby threatening public health (Alexander and Brown, 2000; Chen; *et al*., 2000; Kilpatrick, *et al*., 2006).

Highly pathogenic avian influenza (HPAI) virus, subtype H5N1, was first detected in poultry in November 1996 in southeast China, including Guangdong and Hong Kong where it originated and migratory waterfowls were later infected (Xu *et al*., 1999; Chen *et al*., 2006; Gauthier-Clerc *et al*., 2007). For the first time also, the virus known to be restricted to avian host, cross the species barrier infecting human and several other mammals (FAO, 2007). The outbreak spread through many countries in Asia and Eastern Europe. Scientists believed that migratory birds which became susceptible might spread infections as they overwinter in the tropics (Olsen *et al*., 2006). Predictably, by January 2006 when wintering birds had already begun their seasonal migration to the tropics from Europe, an outbreak was detected in a large commercial poultry farm in Northcentral Nigeria (Adene *et al*., 2006). Earlier report doubted the source of introduction of the virus into the country, but favoured possibility of multiple introductions (Ducatez *et al*., 2006; De Benedictis *et al*., 2007). The index poultry farm, though a large commercial holding was known to acquire multiple species, multi-age birds including point of lay and exotic birds from various trade sources with the attending risk of introducing infection to its flock (De Benedictis *et al*., 2007). That provided preliminary conjectures on the introduction and spread of HPAI H5N1 to Nigeria and the Federal Ministry of Agriculture considered that trade had brought the virus into Nigeria, a view that was also promoted by Birdlife International (Cecchi *et al*., 2008). Hence it was suspected that human activities related to illegal importation of poultry and poultry products into Nigeria from Asia were likely source of transmissions and this held sway until more evidence on the role of migratory birds later emerged (Salzberg *et al*., 2007; Gauthier-Clerc *et al*., 2007; Gaidet *et al*., 2008; Fusaro *et al*., 2009; Snoeck *et al*., 2011; Coker *et al*., 2014).

Ten years after the index case and subsequent outbreaks of avian influenza in Nigeria, it is useful to critically analyze available ecological and genomic evidence describing the risk of introduction and spread of HPAI to Nigeria through migratory wild birds. Following the initial introduction of HPAI H5N1 in the country in 2006, it was followed by a re-introduction that was reported nine years later in 2015 with similar epidemiological pattern as in previous outbreak (Joannis *et al*., 2008; Monne *et al*., 2015; Tassoni *et al*., 2016; Akanbi *et al*., 2016). Recent detection of a new strain of HPAI H5N8 (clade 2.3.4.4) in Nigeria in December 2016 following widespread outbreak in Europe suggests large scale geographical spread through long distance flights of infected birds along the major migration routes connecting Eurasia and Africa (Promed, 2016; FAO, 2017 Lee *et al*., 2017; Pohlmann *et al*., 2017). This also provides more suggestions on the role of migratory wild birds in the introduction of avian influenza virus from Europe in a southwestern direction to Africa. Some of the ecological and molecular epidemiological evidence supporting this hypothesis are discussed.

MATERIAL AND METHODS

In this work, we used a mix-method of mini-review and molecular virology to implement this research. Briefly, we carried out literature search in PubMed, AGORA, HINARI, Google scholar, ScienceDirect, Scopus and ISI web of knowledge with the keywords, avian influenza, and migratory birds as reported with respect to Nigeria, West Africa and globally. Sixty literatures that were more specific were selected for study out of the many results obtained. Technical papers and reports from government and non-government agencies including the Nigerian Veterinary Services Department (Federal Ministry of Agriculture and Rural Development), African Union-InterAfrican Bureau for Animal Resources (AU-IBAR), World Organization for Animal Health (OIE), World Health Organization (WHO), Food and Agriculture Organization of the United Nation (FAO-UN), World Bank and ProMed (International Society for Infectious Disease) were obtained and or tracked on the world wide web (www) and studied for information, alerts, records, documents and data.

Many of the publications examined (over 100) had general information on avian influenza and were screened down to data that were more specific on association between migratory wild birds and avian influenza in Nigeria. The rest of the literatures were reviewed as an extension of the spread, direct and indirect impact including economic losses, wildlife and public health implication of HPAI in Nigeria. In addition to literature search and analysis, opinions were sought through Email interviews from scientists, experts and professionals including veterinarians, epidemiologists, ornithologists, climatologists, wildlife researchers and virologists in Nigeria. These professionals were randomly selected through professional associations and online networks. Their views were assessed viz a viz-published evidence and are included in the discussion. We also conducted phylogenetic analysis of 100 full hemagglutinin gene sequences (obtained from GenBank and GISAID) of H5N1 clade 2.2 and 2.3.2.1c introduced into Nigeria in 2006 and 2015 respectively and sequences of H5N1 isolates from poultry and wild birds from Europe. The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987), the genetic distances between the nucleotide sequences were computed using the Tamura–Nei model (Tamura and Nei, 1993) and bootstrap values of the phylogenetic nodes were calculated out of 1000 replicates (Felsenstein, 1985).

The optimal tree with the sum of branch length = 7.15968210 is shown (Figure 1). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. The analysis involved 78 nucleotide sequences and edited to 28. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1678 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 (Tamura *et al*., 2013).

RESULTS AND DISCUSSION

The role of agro-ecology in the transmission of HPAI

The first laboratory confirmed diagnoses of HPAI H5N1 case in Nigeria was carried out at the National Veterinary Research Institute in January 2006 (Joannis *et al*., 2008) following field and epidemiological investigation by Adene *et al*. (2006). The result of the diagnosis was confirmed and subsequent molecular characterization was carried out at the OIE/FAO Reference Laboratory for Avian Influenza and Newcastle Disease in Padova Italy (De Benedictis *et al*., 2007). While the source of introduction of HPAI into Nigeria was not immediately clear, other scientists simultaneously detected H5N1 from other parts of the country and evidence of multiple introductions in different species of bird from southern and northern parts of Nigeria were described based on distribution of phylogenetic clades (Ducatez *et al.,* 2006; Meseko *et al*., 2007, Fusaro *et al*., 2009). To date, most cases of HPAI recorded in Nigeria since the 2006 incidents were predominantly from the northern part of the country primarily five states of (Kaduna, Kano, Katsina, Bauchi and Plateau). These states are the densely populated poultry producing states in northern Nigeria and are in close proximity to the Hadejia Nguru wetland where migratory birds from Europe are known to winter (Cecchi *et al*., 2008). Over 44 wild bird species, including waterfowls, waders and passerines have been caught in this site during surveillance for avian influenza in wild birds (Gaidet *et al*., 2008: Snoeck *et al*., 2011). Irrespective of the large commercial poultry holdings in Nigeria, 65-80% of the estimated poultry populations are in backyard and rural production system (Adene and Oguntade, 2006). In these villages, backyard and free range poultry, multiple species including waterfowls are kept in poor biosecurity settings. Some of these birds intermingle with resident and migratory wild waterfowls that are frequently sold in live bird markets (LBMs) and large number are transported to southern parts of Nigeria along the poultry value chain (Coker *et al*., 2014). Poultry

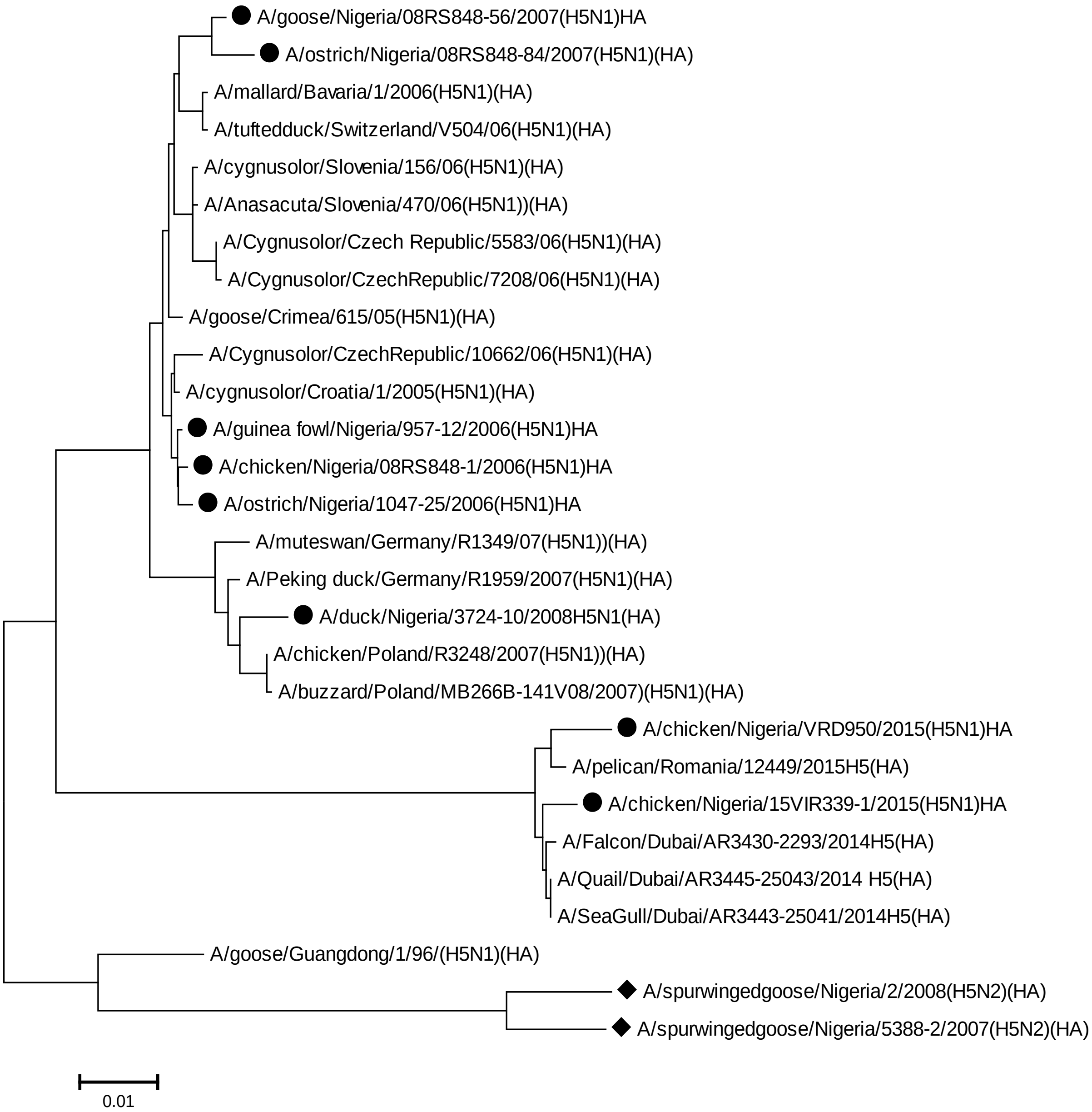

Figure 1: Mid-point-rooted neighbour-joining tree showing phylogenetic relationships between the nucleotide sequences of some HPAI H5N1 from Nigeria, with those of wild birds from Europe (✹-H5 virus from domestic birds in Nigeria, ◆- H5 virus from migratory wild bird in Nigeria)

production within this type of agro-ecology is predisposed to infections. Therefore, birds infected with avian influenza may easily be introduced into LBMs and subsequently farms because no pre-inspection assessment is conducted, with further transmission of infection as was observed in 2015 when the first case of HPAI H5N1 in Lagos was detected in a LBM (Monne *et al*., 2015).

REVIEW

The role of Migratory Waterfowls and Resident Wild Birds in the Transmission of HPAI

Earlier outbreaks of disease due to novel highly pathogenic avian influenza virus of the H5N1 subtype were reported in 1996 in a goose farm in Guangdong Province, China

(Xu *et al*., 1999). Outbreaks of avian influenza H5N1 have also occurred in gallinaceous birds (chicken and turkeys) since 1997 when high mortality were observed in domestic poultry but infection did not affect wild waterfowls (ducks, geese, swans) until 2002 (Ellis *et al*., 2004). However, in May 2005, a major outbreak of HPAI H5N1 was detected in wild birds in Lake Qinghai, which is an important breeding site for migratory birds in China (Gilbert *et al*., 2006). Subsequent outbreaks along migratory bird routes from Southeast Asia, Mongolia through Eastern Europe to Western Europe and in places not associated with domestic bird were observed. Wild birds where thus suspected to play a role in the long distance spread of HPAI H5N1 along these migratory routes (Li *et al*., 2004).

Wild birds, particularly waterfowls of the order *Anseriformes*, including ducks, geese and swans are natural reservoir hosts of all 16 HA subtypes of Low Pathogenic Avian Influenza (LPAI), which are precursors of HPAI (Alexander, 2007). *Charadriiformes* including shore birds, gulls, terns also habour influenza virus but of a different gene pool from *Anseriformes* and the two orders are most important in the long distance transmission and spread of avian influenza (Kawaoka *et al*., 1988; Stallknecht and Shane, 1988), though avian influenza viruses have been isolated from more than 105 wild bird species in nature (Olsen *et al*., 2006).

Waterfowls migrate seasonally to escape extreme winter and move to locations with more favourable conditions, thereafter they move back towards the arctic during spring when there are more vegetation and insects to feed on, and the birds moult and breed (Alerstam *et al*., 2006). Prior to winter migrations, these birds congregate at the breeding sites where different species of adults are mixed with immunologically naive juveniles (under one year), hence predisposing them to avian influenza virus with peak of infection observed just before migration in multiple species, and at concentration areas (Stallknecht and Shane, 1988). Some of the common breeding sites and waterfowls' congregation areas are Southeast Asia, near Lake Qinghai, Northeastern Russia and Siberia. West Siberia lowland is reputed to be the largest wetlands in the world with an area covering 2,745,000 square kilometers (Gilbert *et al*., 2006). Other wetlands are found in Western Russia and Northern Scandinavian. In Europe, wetlands are found along the coast of Denmark, United Kingdom, France and Northern Italy etc. Migratory routes are linked to these breeding and wintering sites depending on prevailing weather. Some breeding and wintering sites are also found around the Scadinavian, Baltic Sea and wetlands in tropical Africa especially West Africa which are reached by long distance migrating birds particularly when winter is severe in Europe (ProMed, 2016).

The earliest record of highly pathogenic avian influenza outbreak in Africa occurred in South Africa in 1961 in migratory common Terns from Europe (Rowan, 1962) and in 2004 HPAI H5N2 was also detected in Ostriches in the country and the epidemiology was closely associated with emergence from LPAI from migratory Egyptian goose (Abolnik *et al*., 2006).

The major and most important wetland in Nigeria is the Hadejia-Nguru, in Northern Nigeria that covers an area of about 3,000 square kilometers enclosing 5 States (Kano, Jigawa, Yobe, Gombe, and Bauchi) where large numbers of migratory species mix with resident wild birds (Snoeck *et al*., 2011). This wetland is also home to birds' sanctuaries including the Dagona waterfowl sanctuary curated as biodiversity hub (Gaidet *et al*., 2008). In a publication by Lameed (2011), 135 species of wild birds including migratory Accipitridae and Palearctic species were observed in this sanctuary.

In the last 20 years in Europe and Africa, most outbreaks of HPAI H5N1 coincide with the annual migration of waterfowls across the globe. Specifically, in 2005 when HPAI H5N1 was detected in domestic poultry in Russia and Kazakhstan, the strain was genetically related to the strain responsible for infection in Lake Qinghai (Lvov *et al*., 2006). Many other species of waterfowl have been shown to harbour HPAI without showing clinical signs as documented in studies from Russia (Lvov *et al*., 2006), China (Chen *et al*., 2006) and Nigeria (Meseko *et al*. 2008) among others. Migratory wild birds are therefore seen to play significant role as long distance transmitters of avian influenza (Hye-Ryoung *et al*., 2012). Their migration from place to place results in the wide geographical spread and distribution of influenza virus and consequence contact of infected migratory birds may transmit or bridge infections to resident domestic birds and poultry (Vakuru *et al*., 2012; Kaplan and Webby, 2013). Some of these wild birds have been caught in Nigeria; Bayelsa State (a homing pigeon from Britain), Sokoto State (a falcon from Finland) and Benue State (a dead raptor from Germany with wing tag number 248) (Obi *et al*., 2008). A migratory raptor from Finland with ring tag number 258211 was also caught by hunters in wetland environment of Taraba State in 2009 and upon pathological and virological examination was found to be positive for Newcastle disease virus that shares similar clinico-pathological manifestation with avian influenza and could have been transmitted to domestic birds (Meseko and Moses, 2012). More recently in 2015 in the course of the HPAI epidemics in Nigeria, a migratory osprey from Germany was caught in Kebbi state with ring tag number SA 03209 (Plate 1).

Plate 1: Migratory osprey from Germany caught in Kebbi State in February 2015 with ring number SA 03209 (Courtesy of Veterinary Department, Kebbi State Ministry of Agriculture)

In 2008 during a surveillance programme in wild birds in Europe, Middle East and Africa, Gaidet and co-workers (2008) detected avian influenza subtype H5N2 bearing a highly pathogenic (HP) Haemagglutinin in healthy birds that were sampled in Nigeria. In a similar discovery, low pathogenic avian influenza subtype H5N2 was also detected in wild migratory fowls around the Hadejia-Nguru wetland not far from Kano in 2008 (Snoeck *et al*., 2011). These studies confirmed that both low and high pathogenic viruses were present and introduced by wild birds into Nigeria landscape especially around the wetland areas (Gaidet *et al*., 2008). Interestingly, Coker *et al*. (2014) also found the LPAI H5N2 in domestic waterfowl sold in Live Bird Market in Southwest Nigeria few years later.

In the quiescent period spanning 2008 to 2014, no infection of highly pathogenic avian influenza was reported either through passive or active surveillance in Nigeria prompting self-declaration of HPAI H5N1 disease free status at the World Organization for Animal Health General Session in 2013 (FMARD, 2013; OIE, 2013). By early January 2015 described as the cold harmattan months in the country, incidence of high mortalities in poultry were confirmed to be caused by HPAI H5N1 but of a different clade 2.3.2.1c being detected for the first time in Nigeria (Monne *et al*., 2015). That period coincides with adverse winter in Europe, when migratory waterfowls move southward to the not so cold or warmer environment with the potential of carrying infectious pathogens along the route and into places where they epidemiological scenarios related to avian influenza in Nigeria occurred in 2006-2008 and re-occurred 2015-2016 as shown in published temporal prevalence of HPAI H5N1 with typical epidemic curves describing patterned distribution of cases (Fasina *et al*., 2009: Ekong *et al*., 2012; Akanbi *et al*., 2016). Interestingly, the strain HPAI 2.3.2.1c that caused infections in poultry in Nigeria in 2015 was eventually detected in a migratory birds and also poultry in the Middle East and Europe along migratory routes (Yuhai *et al*., 2016). The epidemiological deduction is that avian influenza is usually introduced at the end of the year around November to December and local transmission aggravates infections through increase movement and trade in poultry and poultry products during the period (Cecchi *et al*., 2008; Monne *et al*., 2015).

In recent episodes in Europe, enunciating the role of migratory birds in the epidemiology of HPAI, another subtype (H5N8) caused unprecedented outbreaks in the autumn of 2016, and was detected mostly in 36 species of wild and migratory birds (Pohlmann *et al*., 2017). In following the hypothetical pattern of outbreaks along the route of migratory birds fly ways from Europe to the Middle East and Africa, infections in Europe were first confirmed in Germany which also recorded highest number of cases. Shortly after, reports were received from Israel, Tunisia and Egypt, and eventually in Nigeria following pattern of wild bird migration. Many other countries in Africa (Uganda, Zimbabwe and South Africa) have also reported cases of H5N8 in 2017. The outbreak in Uganda was first observed in migratory birds (*Chlidonias leucopterus*) on the shores of Lake Victoria which eventually spill over to domestic poultry (ProMed, 2016; FAO, 2017).

The HPAI H5N8 viruses detected in Nigeria were from backyard poultry and apparently healthy waterfowls on sale in LBM in Kano city. This repeatedly affirms suggestions of stopover. This ecological and an agro-ecological link within the high poultry density areas around Hadejia-Nguru, the largest wetland and wild bird sanctuary in the country. Though it has been difficult to detect HPAI first in wild bird before spill over events to domestic poultry in Nigeria, targeted virological surveillance in these hotspots should be considered as this region appears to play significant role in the context of migratory birds' introduction of HPAI into Nigeria (Cecchi *et al*., 2008).

Molecular Epidemiology

Molecular evidence linking HPAI H5N1 detected in migratory birds and isolates from domestic poultry in Nigeria has been described previously by Fusaro *et al.* (2009). This was based on the result of molecular characterization of avian influenza H5N1 isolated in an apparently healthy waterfowl that was sampled in the course of passive surveillance in live bird markets in Gombe state Nigeria in 2008 (Meseko *et al*., 2010). Though many authors have also suggested that migratory birds could play a role in the introduction of avian influenza in Nigeria, it has often been as conjectures while not ruling out trade sources. (Ducatez *et al*., 2006; De Beneditis *et al*., 2007, Fasina *et al*., 2009)

Molecular and phylogenetic analysis of the isolate from healthy waterfowl in Gombe further showed that the virus clustered and shared closest relationship with HPAI H5N1 clade 2.2.3 detected in migratory birds known to have originated from Europe and Middle East (Fusaro *et al*. 2009). Sequence analysis of the gene segments showed highest similarity at nucleotide level with a migratory goose- Cygnus olor/Czech Republic with 99.3% HA; 99.8% NA and 100% M genes homology. The virus also showed high similarity with H5N1 strain that circulated in Romania and Eastern Europe earlier in 2007 (Fusaro *et al*., 2009).

As previously inferred in the agro-ecological importance of wetlands in the introduction of avian influenza to Nigeria, samples from apparently healthy wild waterfowl that were collected at the Hadejia-Nguru wetland (Dagona waterfowl sanctuary) were found positive for HPAI H5N2 with H5 sequences revealing highest homologies with H5N2 LPAI isolate A/mallard/Bavaria/1/2005 (98.2%) (Gaidet *et al*., 2008). One of two birds was fitted with satellite telemetry after sampling and tracked for 47 days. Observations showed that though the bird was infected with HPAI, it survived and migrated for 655 km to another country before it was lost to tracing. In a similar study, LPAI H5N2 was found in three spur-winged geese (*Plectropterus gambensis*) in the same Hadejia-Nguru wetlands between March and April 2008 (Snoeck *et al*., 2011). Phylogenetic analysis of the H5N2 virus showed that all the genes, except NS were most closely related to genes recently found in wild and domestic birds in Europe. The authors concluded that the presence of LPAI H5N2 in these wild waterfowls in the Hadejia-Nguru wetlands, where wild birds and feral resident birds frequently interact, provide ample opportunity for infection across species and generating gene reassortments. This assertion was reinforced when LPAI H5N2 was detected in apparently healthy domestic waterfowls in a LBM in Ibadan (Coker *et al*., 2014). The phylogeny of the virus from LBM showed a clustering with H5N2 viruses previously detected in Africa with an indication of virus transmission in the pattern from wild migratory birds to domestic waterfowls. Researchers have shown that HPAI viruses arise through mutation after LPAI viruses of H5 and H7 subtypes were introduced into poultry (Alexander, 2007; Kim *et al*., 2009). This is an indication that LPAI that has been detected more than once in poultry in Nigeria has the potential through antigenic shifts and drifts for reversal to virulence and should be carefully monitored (Alexander and Brown, 2009; Coker *et al*., 2014).

Recently in 2015, following reports of outbreaks in Europe and Middle East another strain of H5N1 was isolated in domestic poultry in Nigeria having 99% full genes homology with isolates earlier detected in China and Middle East in 2013 (Monne *et al.*, 2015, Shittu *et al*., 2017). The phylogenetic tree that was estimated for the HA gene demonstrated that the H5N1 virus from Nigeria A/chicken/Nigeria/15VIR339-2/2015) clusters within genetic clade 2.3.2.1c. In the publication by Monne and colleagues (2015), it was also observed that genetic clustering between strains from Nigeria and Asia has an unknown epidemiological link between the two regions. In this paper, we suggested that migratory birds that flew from Asia to Europe through Russia and then fly to Africa were probably responsible for this link (Marchenko *et al*., 2015). Our study further demonstrate this when recent GenBank BLAST analysis of A/duck/Nigeria/3724-10/2008 H5N1(HA) detected in apparently healthy duck in Gombe LBMs returned 99% homology (99-100% query cover) with over 20 wild birds (Cygnus olor, swan, Peking duck, etc) from Czech Republic, Germany and Poland. The Phylogenetic tree (figure 1) of selected nucleotide sequences from Nigeria and Europe showed clustering of some HPAI from Nigeria with those of wild birds from Europe as has been variously described by other authors (Gaidet *et al*., 2008, Fusaro *et al*., 2009; Snoek *et al*., 2011; Coker *et al*., 2014; Monne *et al*., 2015).

Conclusion

Our literature search and analysis of nucleotide sequences have provided additional evidence that migratory birds are primary sources of introduction of HPAI to Nigeria. Feral birds and trading in local fowls may bridge transmission to domestic poultry. In the light of this understanding, routine risk based sentinel surveillance in hotspots and in wild and domestic fowls is recommended for early detection and control of avian influenza thereby saving economic losses and mitigating public health emergencies.

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