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REVIEW ARTICLE

Newcastle disease in Nigeria: epizootiology and current knowledge of circulating genotypes

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Abstract Over the years, Newcastle disease (ND) has defied all available control measures. The disease has remained at the forefront of infectious diseases afflicting poultry production after avian influenza. Despite the continuous global use of million doses of ND vaccine annually, the causative pathogen, avian paramyxovirus type 1 also known as Newcastle disease virus (NDV) has continued to evolve causing, even more, a threat not only to the unvaccinated but the vaccinated flocks inclusive. The disease has been well studied in the developed countries where the virus is found in circulation. However, limited information exists on the epizootiology and circulating genotypes of the virus in developing countries where the majority of the flocks are raised on the extensive management system. Identification of virulent NDV in apparently healthy free-range ducks in this system calls for concern and pragmatic approach to investigate factor(s) that favour the virus inhabiting the ducks without clinical manifestation of the disease. Recently, novel genotypes (XIV, XVII, and XVIII) with peculiarity to West and Central African countries have been discovered and due to lack or poor surveillance system possibility of hitherto unreported genotypes are likely. This review elucidates and discusses available literature on the diversity of the circulating NDV genotypes across the West Africa countries and the epizootiology (molecular) of the disease in Nigeria with the view of identifying gaps in knowledge that can assist in the development of effective vaccines and control strategies to combat the peril of the disease.

Keywords Epizootiology · Newcastle disease · Genotypes · Nigeria · West Africa

Introduction

The first outbreak of Newcastle disease (ND) in poultry can be traced back to 1926. This occurred at two diverse geographical points on the world map, Newcastle-upon-Tyne, England [26] and the Island of Java, Indonesia [55]. However, there are strong suggestions that ND outbreaks might have occurred earlier than reported by Doyle and Kraneveld as contained in the paper written by Macpherson [60] who drew attention to the poem titled “Call nan Cearc” (The loss of the hens) written by John Campbell on his conviction that the outbreak described in the poem which occurred in 1898 in Western Isles of Scotland was caused by ND. This review will toe the line of reports of Doyle and Kraneveld since the description in the poem could resemble that of “fowl plague” (avian influenza) which was ravaging poultry around that time in Italy [87]. The disease has continuously decimated and threatened the growth of poultry production, particularly in developing countries [9].

ND is a highly infectious disease with great economic losses, making it one of the “notifiable diseases” on the World Organization for Animal Health (OIE) list (http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.14_NEWCASTLE_DIS.pdf). Designation of pathogens as “notifiable diseases” requires immediate and

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prompt reporting to the relevant authorities to curtail the spread. Outbreaks of ND occur across the globe on a daily basis, especially in developing countries where the disease is enzootic with only a few countries reporting to the OIE. In a 12 year study (January 2000–December 2011) conducted to examine the frequency of reporting of ND outbreaks in 54 African countries, only 40.7 % was discovered to have always reported to OIE [38]. According to OIE (www.oie.int/wahis_2/public/wahid.php/), outbreaks of ND have been reported at one time or the other in some countries from Africa, South America, Middle East, Europe and Asia from 2005 to 2015.

From the time of the first reported outbreaks in 1926, four panzootics of ND have been documented [9, 45] and the possibility of another panzootic is imminent [67]. Based on clinical signs in infected birds, five pathotypes of NDV isolates have been identified, namely: neurotropic velogenic, viscerotropic velogenic, mesogenic, lentogenic and asymptomatic. Pathogenicity for chickens varies significantly, ranging from asymptomatic (no apparent disease) to severe. In severe cases, it manifest as respiratory and/or neurological disease culminating in 100 % mortality of infected flocks in the case of the velogenic strains. Assessment of economic impact due to ND is not only limited to high mortality recorded from the outbreaks and the cost of control measures but also on the trade restrictions placed on the localities where outbreaks have been reported [8, 9, 37].

Poultry production in Nigeria

The poultry industry in Nigeria is the most capitalized among the Agricultural sectors in the country and contribute the largest to the economy next to the oil industry [3, 27]. About 65–75 % of the populace depends on agriculture and agro-based businesses for their income [http://www.cenbank.org/OUT/PUBLICATIONS/REPORTS/RSD/2009/CBN Annual report for the year ended 31st December 2007–Executive summary. Pdf; 13] with poultry identified as a major source of national income that provides about 9–10 % of the nation's gross domestic product (GDP) worth $250 million [13]. In Nigeria, an increase in poultry production was actually noticed in the early 1980s. At this time, subsidy was introduced on day-old chicks (DOC) and feed ingredients by the government. Recently, the government supported small scale poultry farmers with DOC and other incentives at highly subsidized rates to boost poultry production through the Agricultural Transformation Agenda. These initiatives have encouraged several people, including civil servants, housewives, and artisans to embrace poultry production by keeping birds as part or full-time commercial ventures. As practiced in most developing economies, poultry production systems are generally categorized into two major groups, namely; subsistent (free-range) and commercial [4]. Based on biosecurity practices, FAO further categorizes poultry production systems into 4 sectors viz; sector 1 with high, sector 2 with high to moderate, sector 3 and 4 with low biosecurity. In Nigeria, sector 4 (free-range poultry) accounts for over 80 % of the poultry population [3]. Different species of poultry, including layer, cockerels, guinea fowls, ducks, turkeys, and pigeons are raised by the operators of the various systems. In the 1990s, Japanese quail (Coturnix coturnix japonica) was introduced into Nigeria by National Veterinary Research Institute, Vom to diversify the poultry subsector and provide alternatives to poultry consumers in terms of meat and eggs [41]. Consequently, Japanese quail products have received general acceptance and patronage from poultry consumers across Nigeria due to its nutritional, economic and perceived medicinal values [16]. However, these production systems, especially the subsistence are faced with myriads of challenges amongst which disease is topmost. Notable among the diseases afflicting the industry are Infectious bursal disease (Gumboro), Chicken anaemia virus, Infectious Laryngotracheitis, Fowl pox, Salmonellosis, Chronic respiratory disease, Marek's Disease, ND, Egg drop syndrome, Infectious bronchitis, Avian Influenza and so on [3, 31, 32, 50, 76, 81, 84]. The sustainability of this subsector is being threatened as a result of incessant outbreaks of ND in unvaccinated flocks and sporadically in vaccinated flocks [5, 102].

The first report of ND in Nigeria was in 1952 [44], thereafter several cases have been reported in commercial, rural scavenging, captive and free-living wild birds making it enzootic across the entire country [5, 28, 31, 40, 48, 70, 72, 74, 82]. Reports have shown that ND was ranked first among other diseases affecting the poultry industry [3, 39]. Economic and financial losses as a result of incessant ND outbreaks in Nigeria are not being regularly quantified. An estimated 78,526 outbreaks of the disease were reported in 2008 across Nigeria with an estimated financial burden of 8.9 billion naira for local chickens alone [29]. This report is an underestimation of financial implications of annual incidence of ND across the country in terms of the magnitude of unreported outbreaks occurring in remote areas of the country.

Biosecurity and vaccination are veritable tools that are used in combating the disease. Various types of vaccines are in use in the country for the control of ND menace. Notable among the vaccines are live attenuated Lasota and Hitchner B1 strains which are commonly used in the commercial poultry. In addition, inactivated oil-emulsion vaccines are often used by some farmers, which come as a single package or in combination with other poultry agents.

Table 1 Current vaccination schedule for Newcastle Disease (ND) in Nigeria

S/N	ND vaccine	Age of vaccination	Route of inoculation
1.	Live attenuated Hitchner B1	Day old at the hatchery	Intra-ocular through spraying
2.	Live attenuated La Sota	1–2 weeks	Oral through drinking water
3.	Live attenuated Komarov	7–10 weeks	Intramuscular through injection
4.	Inactivated oil-emulsion NDV	16–18 weeks	Intramuscular through injection

The vaccination regimen (Table 1) is routinely undertaken in commercial poultry. However, vaccination in free-range poultry system is barely performed due to the large dosage presentation of conventional ND vaccines, and maintenance of cold chain. On the other hand, thermostable NDV-I_2 and V4 vaccine strains have been successfully introduced into Nigeria and used in combating ND threat for free-range chickens with good protection level [68, 78]. The free-range chickens have been implicated in harbouring velogenic strains of the virus [5, 28, 62] which have been considered a threat to the commercial poultry.

Definition of Newcastle disease

In general terms, infection of birds with any strain of NDV may be referred to as ND but because ND is a "notifiable disease" to the OIE the appropriate definition must, therefore, be followed. According to the definition by OIE (http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.03.14_NEWCASTLE_DIS.pdf), ND is an infection of birds caused by APMV-1 that fulfils any of the following criteria for virulence (a) Exhibition of intracerebral pathogenicity index (ICPI) value of 0.7 or greater in day-old chicks or (b) Presence of at least three basic amino acids (arginine or lysine) at between residue positions 113 and 116 of the C-terminus of the F2 protein and phenylalanine at position 117, which is the N-terminus of the F1 protein. In cases where the pattern of amino acid residues observed contradicts what was stated above, characterization of the virus by intra-cerebral pathogenicity index (ICPI) test would be performed. Identification of the amino acid residue positions starts from the N-terminus of the amino acid sequence deduced from the nucleotide sequence of the F0 gene with 113-116 corresponding to residues −4 to −1 from the cleavage site [9].

Molecular determinant of virulence

The molecular basis for pathogenicity can be deduced from the presence of multiple basic amino acids at the cleavage site of the fusion protein or ICPI index of 0.7 or greater than in day-old chickens [9].

Newcastle disease virus

NDV is classified into the genus *Avulavirus*, subfamily *Paramyxovirinae* and family *Paramyxoviridae* (http://www.ictvonline.org/virusTaxonomy.asp). Based on serological tests and complete genome sequence, APMVs have been officially classified into 12 serotypes designated as APMV-1 to 12 with NDV categorized into APMV-1 [7, 10, 65, 106]. The virus genome has a negative-sense, non-segmented, single-stranded RNA with a genome length of approximately 15,200 nucleotides (nt). Isolates of early 1930–1960s have a genome size of 15,186 nt while those of late 1960s have 15,192 nt and 15,198 nt for those of Class I isolates from waterfowls [21, 63]. The genome codes for six genes encoding seven proteins, namely: nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase protein (HN), large polymerase protein (L), and an additional protein V that is expressed by RNA editing of P mRNA [56]. The HN and F proteins are two spike projections on the envelope of the virus and the F-gene in addition to the HN and L genes have been identified as a major determinant of virulence [23, 47, 86]. The genome size is a multiple of six nt, referred to as the 'rule of six' that makes efficient replication occur [21, 24, 54]. Though, the virus exists as a single serotype antigenic and genetic diversities have been identified among the isolates across the globe [93].

Full genome sequences of APMV-1 strains of the differently identified genotypes circulating across the globe have been published [17, 20, 34, 52, 59, 107]. In West Africa, however, only four complete genome sequences of the virus circulating in this sub-region are available in the sequence repositories (GenBank, DDBJ, and EMBL) as of 30th May, 2016. In addition, complete genome sequence of APMV-1 representing genotypes XIV and XVII from Nigeria has just been published [97, 98]. The paucity of information on the circulating genotypes in the sub-region is a limitation in the development of effective control measures.

Newcastle disease outbreaks in Nigeria

In general, all avian species are susceptible to ND infection, but the chickens are the most affected in terms of severity of the disease [10]. In chickens, 100 % mortality

Table 2 Seroprevalence study of ND in local chickens and live bird markets Nigeria

Location	Year	Prevalence %	References
Ogun	2006	100 (180/180)	[85]
Plateau	2009	51.9 (627/1208)	[69]
Jigawa	NA	38.8 (72/250)	[109]
Bauchi	NA	56.3 (169/300)	[71]
Nassarawa	2011	28.1 (289/1030)	[92]
Nassarawa	2011–2012	28.7 (359/1250)	[43]
FCT (Abuja)	NA	17 (34/200)	[2]
FCT (Abuja)	NA	57 (228/400)	[14]
Zamafara	NA	32.5 (164/504)	[49]

has been recorded with an infection involving velogenic strains [37]. Ducks and quails are resistant to the disease, but recent studies have shown their susceptibility even though the morbidity and mortality are lower than chickens [58, 80]. In Nigeria, ND outbreaks have been reported in free-range local and exotic chickens, guinea-fowls, wild and captive birds, quail, dove, mallard duck, ostrich, turkey, vulture, eagle, sparrows, crows, parrot [6, 39, 40, 48, 72, 78, 83] (Shittu et al. 2016 unpublished data).

Epizootiology of ND in Nigeria

Newcastle disease is a global disease with a presence in six out of the seven continents [66]. In Nigeria, the disease has been detected in all the agro-ecological zones of the country (NVRI unpublished data, 2006). Over the years, epizootiology of ND in Nigeria has been based mostly on conventional methods viz: serological [6, 69, 71, 75, 83, 85, 92] and virus isolation with biological characterization [5, 28, 40, 48, 62, 70, 74, 96]. Recently, however, attempts were made by using molecular techniques at defining the genotypes and molecular epidemiology of the circulating virus in West and Central Africa including Nigeria [19, 99, 101–103, 108].

Using serological methods [haemagglutination-inhibition (HI) or Enzyme-linked immune sorbent assay (ELISA)], antibodies to NDV have been detected in different species of poultry across the country [11, 12, 30, 61, 75, 89]. Over the decades, seroprevalence studies conducted in different locations in Nigeria on birds kept on the extensive production system showed diverse ranges from 38 to 74.3 % [72]. In a more recent seroprevalence study, the figures have not considerably changed (Table 2). There is no doubt to the fact that ND is enzootic in Nigeria, deductions made from serological survey data, therefore, needs to be interpreted with caution. Most of these studies were conducted in or near urban areas. Commercial backyard poultry in these areas raised on semi-intensive system are mostly vaccinated against ND. These birds are sometimes allowed to commingle with the free-range scavenging birds. In another scenario, “spent” hens from commercial poultry which are regularly vaccinated are procured and raised along with the free-range birds. These scenarios make the deductions of the true prevalence from these studies biased due to the confounding factors associated. This may explain the differences in seroprevalence from the same epidemiological units (Table 2). It is, therefore, envisaged that future survey for NDV should be aimed more at virological and genetic characterization rather than serological which provide limited information especially in terms of pathotypes and genotypes.

As shown in Table 3, retrospective studies of clinical case report covering 9–10 years conducted in some northern states of Nigeria identified ND as most often diagnosed disease with a prevalence of 36.7 % [15] and 52.2 % [91] in Borno; 29.1 % [110], 32.3 % [90] and 33.2 % [73] in Kaduna; 14.1 % [79] in Kwara and 55.5 % [57] in Gombe. Also, an isolation rate of 21.0 % was reported in a limited epizootiological study involving apparently healthy birds from four different species in two live bird markets (LBM) in Southwestern Nigeria [96]. In a 3 year prospective study conducted in southeastern Nigeria involving a clinical and laboratory-based test, ND outbreaks were observed to peak during the dry harmattan

Table 3 Retrospective study of ND in Nigeria

Location	Year	Specific rate (%)	References
Maiduguri, Borno state	2004–2012	36.7 (851/2317)	[15]
Maiduguri, Borno state	2000–2009	52.2 (2427/4647)	[91]
Zaria, Kaduna state	1990–1999	32.3 (812/2513)	[90]
Zaria, Kaduna state	2003–2012	29.1 (868/2983)	[110]
Mando, Kaduna state	1996–2005	33.2 (1050/3164)	[73]
Ilorin, Kwara state	2000–2009	14.1 (517/3655)	[79]
Gombe state	2004–2013	55.5 (5531/9970)	[57]

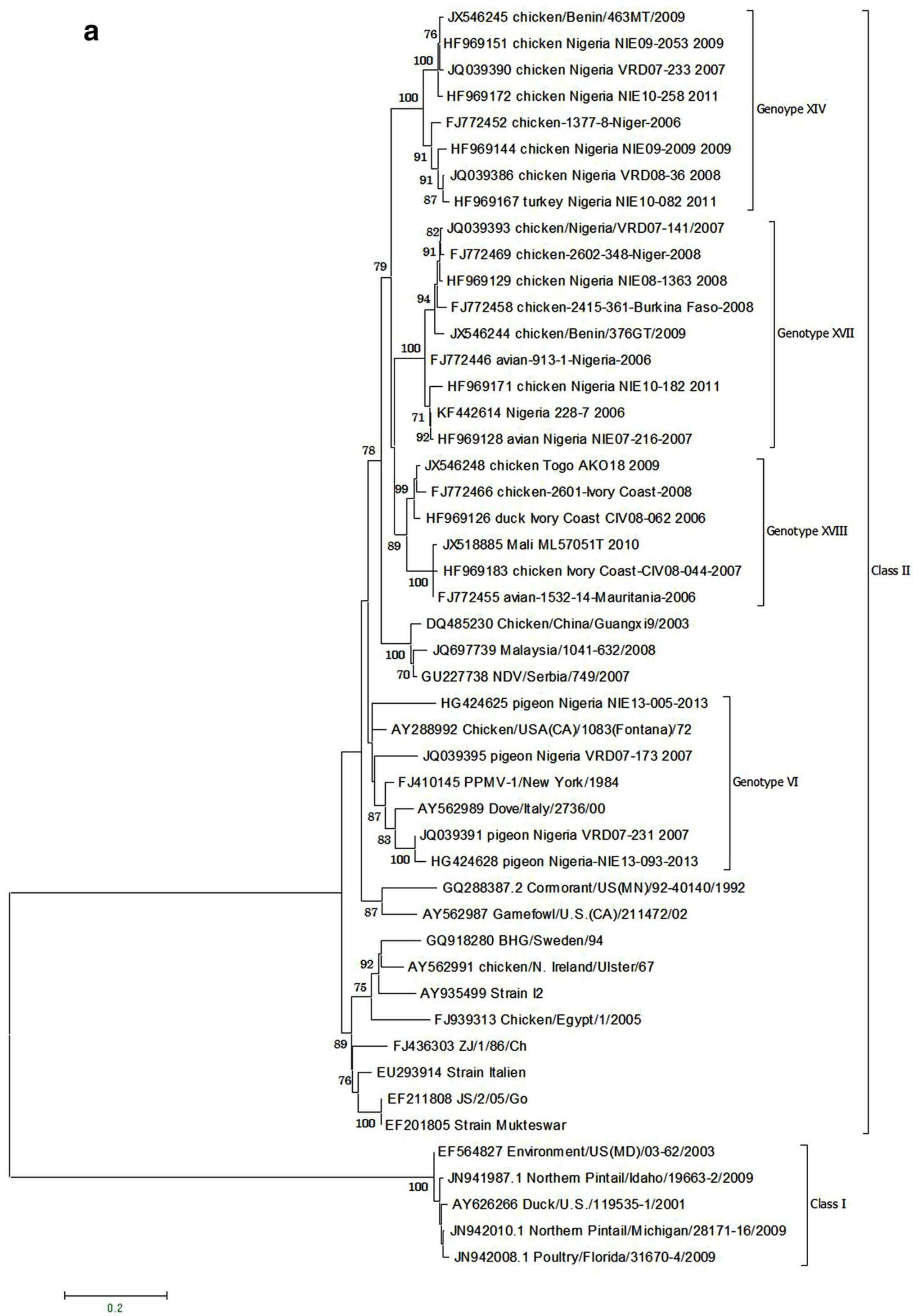

Fig. 1 NDV isolates Maximum Likelihood Phylogenetic tree of the complete fusion gene (1662nt). **a** Phylogenetic tree of Nigerian isolates and other published West African NDV sequences. **b** Phylogenetic tree of NDV genotypes reported in Nigeria with the exception of genotype II (No complete fusion gene available in the GenBank from Nigeria). Sequences from the GenBank are indicated by their accession numbers. The two genotype IV sequences are yet to be deposited in the sequence repository. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model [105]. The tree with the highest log likelihood (−10,045.3536) is shown. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated. At the nodes, only bootstrap values greater than 70 % are shown. Scale bar represents number of substitutions per site

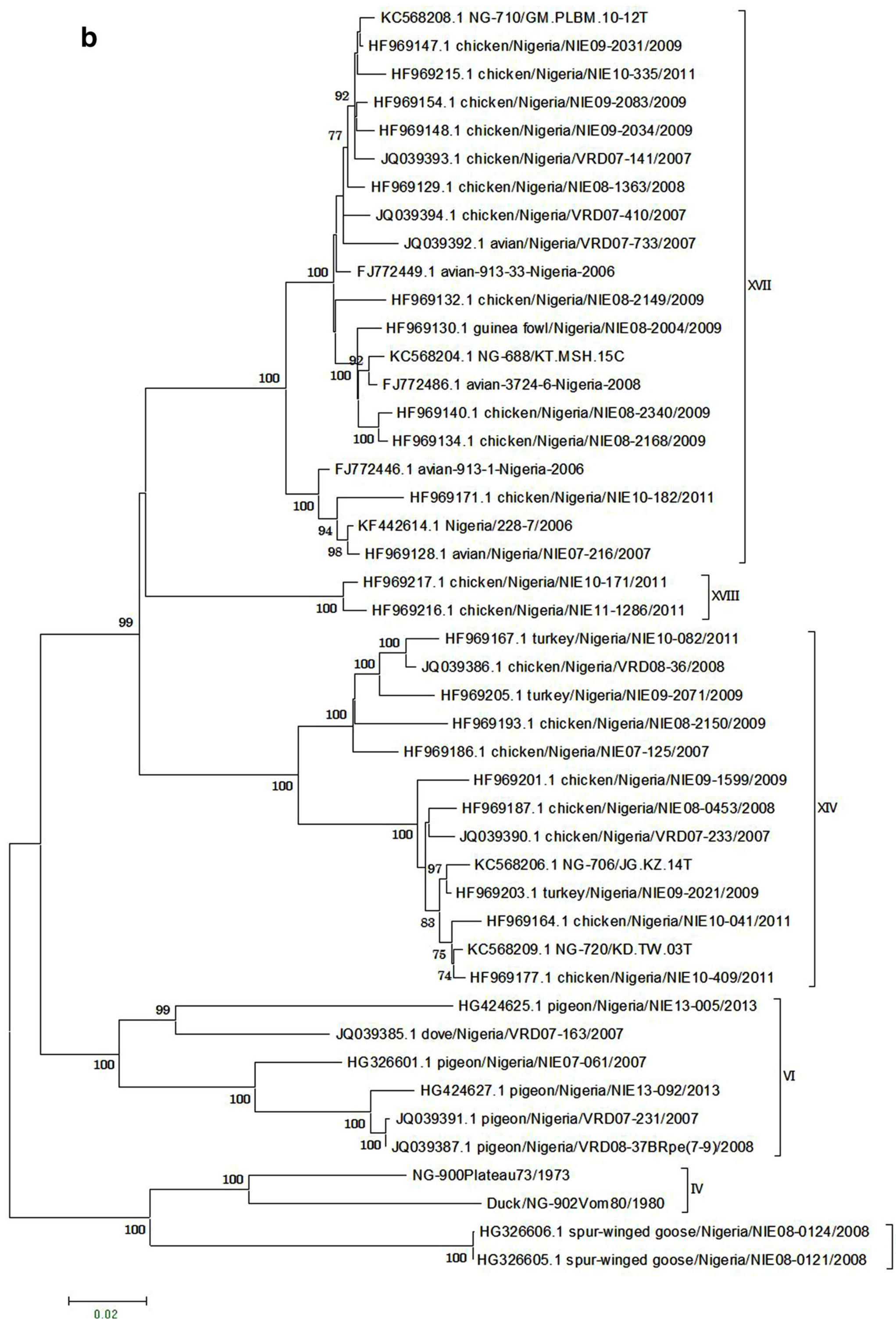

Fig. 1 continued

period (November to February) with another marginal peak recorded during the height of the rainy season (June to July) [77]. In other reports, high prevalence of ND outbreaks was also observed mostly in the dry harmattan period [57, 64, 69, 73, 77, 90, 110] in which stress and cold associated with the weather are said to be responsible for this occurrence [64, 77]. In addition, movements of birds across different parts of the country are witnessed around this time in preparation for the festive period (Christmas and Easter). Likewise, the role of LBM in the perpetuation of the virus cannot be ignored as recently reported [96] which may explain the sporadic outbreaks experienced outside the peak period. Prior to the onset of the raining season in Nigeria, some farmers sell off their poultry and the proceeds are used to procure seeds for the farming season. In some instance, live birds are bought from LBM or poultry vendors in exchange as a gift. Such birds when not immediately needed are introduced into pre-existing flock if any, or left to scavenge in the neighbourhood. These birds might be incubating the virus and shed into the environment. The introduction of seemingly healthy, but inadvertently infected new bird to an existing flock has been reported to result in an outbreak of ND and highly pathogenic avian influenza (NVRI unpublished data, 2006).

Also, reports of isolation of virulent NDV from apparently healthy birds have been documented [18, 22, 28, 62, 99]. In all of these reports, free-range local chickens and ducks were involved. The perpetuation of velogenic strain in apparently healthy birds, therefore, poses a threat to commercial poultry. Future work should be designed to identify the natural genetic resistance gene possessed by these local indigenous birds that enable it to harbour the virus with subclinical infection. Identification of such gene(s) may help to develop resistant chickens through reverse genetics that can help to combat Newcastle disease in both free-range scavenging and exotic commercial chickens.

Unlike other NDV genotypes circulating in other parts of the World, little is known about the genotypes circulating in West Africa. Three new genotypes XIV, XVII and XVIII and two sub-genotypes (Fig. 1a) each has been described and are responsible for the current ND outbreaks being experienced across the sub-region. These genotypes and sub-genotypes are in circulation and have not been detected elsewhere outside the sub-region, making them indigenous [19, 95, 99, 101–103, 108]. All NDV genotypes that have been reported to be circulating in Nigeria are shown in Fig. 1b. Genotype IV was discovered in uncharacterized archival samples in our laboratory dating back to 1980 and 1973 (Shittu et al. 2016 unpublished data). Though, this genotype has not been detected from any of our current surveillance (active and passive) activities. The existence of the genotype has been reported to have ceased to be in circulation across the globe since 1989 [66]. Genotype VI viruses are characterized by pigeon paramyxoviruses (PPMV) which are variants of NDV. They have been reported to be in circulation from 1970 to 1980 and are responsible for the third panzootic [10]. The virus has been reported in different West and Central African countries, including Nigeria with sub-genotypes VIg, h, and i being in circulation [100, 108]. Genotypes I and II were discovered in free-range and commercial poultry respectively, with close relatedness to NDV-I2 and LaSota vaccines [76] (Shittu et al. 2016 unpublished data).

Globally, genotype VII has been reported to be responsible for outbreaks in other countries of the world and current panzootic [66] with no report yet in West Africa. Although, the presence of the genotype has been established elsewhere in Africa, namely; Egypt [88], Libya [51], Sudan [42], South Africa [1], Mozambique [35], and Ethiopia [33].

Challenges and way forward

Prompt diagnosis is the hallmark of disease prevention and control. Unfortunately, as observed in the literature reviewed, most prevalence reports on ND diagnosis in Nigeria have been based on clinical signs and post mortem lesions [57, 91]. The diagnoses are rarely confirmed using laboratory-based diagnostic methods (virus isolation and pathotyping) knowing well that NDV infection is not pathognomonic; this will present unreliable results as revealed by our laboratory data (Shittu et al. 2016 unpublished data). Enzootic status of ND can be upheld based on the continuous circulation of velogenic strains of different genotypes reported to be in circulation in both apparently healthy, wild bird and sick/dead birds in live bird markets, free-range and commercial poultry [19, 98, 99, 102, 108]. As of 30th May 2016, only 8 out of the 18 countries constituting the West Africa sub-region have sequences of NDV in the sequence repositories. In contrast to thousands of available sequences of genotype VII, which have been identified as responsible for the fourth ND panzootic [67]. Having more sequences will aid in understanding the evolutionary dynamics of the circulating strains which will help in the design of effective control of the disease. It is interesting to note that, no reports of genotype VII have been documented in West Africa in spite of the transborder movements of poultry and poultry products which take place legally and illegally across the regions and continents. In addition to migration of wild birds which have been identified as possible sources of introduction of avian influenza [36]. The none detection of this genotype could be as a result of poor surveillance system which exists in Nigeria as well as the sub-region as a whole. Initially, due to the genetic relatedness of the currently circulating genotypes indigenous to West Africa, earlier studies on NDV genotyping in West Africa, grouped the viruses to lineage 5 and 7 (genotype XIV and VII) [19, 94, 99]. The classification was later streamlined to genotypes XIV, XVII, and XVII with the existence of the unified nomenclature for the classification of NDV as proposed by Diel et al. [25] and expanded by Snoeck et al. [101]. Movements of poultry and poultry products within and outside states and the sub-region have assisted in the spread of the disease. This is evident from the genetic relatedness shared among the isolates from different locations across the country. Solomon et al. [103] reported sequence similarities of 99.3–100 % among NDV isolates from within and outside Nigeria. Surveillance activities are to help in early detection and the establishment of effective preventive and control strategy. It is obvious that surveillance for infectious diseases has been lacking which has favoured the spread of these genotypes across many countries in the sub-region largely due to trade and movement of poultry and poultry products.

Concluding remarks

Since the identification of novel genotypes of ND from some West and Central Africa poultry population [19, 99] information on the circulating genotypes has increased. Though this information is still limited, it however provides insight into the diversity of ND across the sub-region. For effective control strategy to be implemented, identifying the various genotypes in circulation is the first step in achieving the desired goals. The current vaccination program in Nigeria and the sub-region at large needs to be reviewed to accommodate the current circulating genotypes. Though, few studies have been carried out to assess the protection level of the currently circulating NDV genotypes (XIV and XVII) [94, 104] with the exception of genotype XVIII against Lasota and V4 vaccine strains. The vaccination regimen in Nigeria includes the use of Komarov (Table 1) for the initially primed chicks at 7–10 weeks of age. The protection level of this vaccination schedule should be assessed with the circulating strains. In addition, the clamour for a genotype-matched vaccine has been in the news lately with several successes recorded using genotype VII [46, 53, 111]. It may therefore be imperative to develop a genotype-matched vaccine against the predominant genotype in West Africa to ameliorate the vaccination program.

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