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Occurrence and Molecular Characterization of Newcastle Disease Virus Strains in Chicken Flocks in Ibadan, Nigeria: Implications for Vaccine Strain Compatibility

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ABSTRACT

Newcastle Disease (ND) poses a significant threat to poultry health and economic stability in Nigeria, with outbreaks affecting both smallholder and commercial farms. Despite vaccination efforts, the persistence of ND outbreaks is attributed to the genetic diversity of circulating Newcastle disease virus strains, which often differ from vaccine strains. This study investigated the occurrence and molecular characteristics of ND virus in chicken flocks within Ibadan between June and September 2024. A cross-sectional study was conducted by collecting samples from chickens that were tentatively diagnosed as ND at Poultry Diagnostic Centers in Ibadan. The presence of ND virus was confirmed through haemagglutination and haemagglutination inhibition tests, followed by molecular analysis using reverse transcription-polymerase chain reaction (RT-PCR) targeting the F-gene. Genetic sequencing and phylogenetic analysis assessed the relationship between detected strains and available vaccine strains. Out of 297 poultry cases submitted for diagnostic evaluation, 26 were suspected to be ND based on clinical signs and pathological findings, yielding an occurrence rate of 8.8%. Following haemagglutination and haemagglutination inhibition tests using ND-specific antiserum, 12 samples (4.04%) tested positive for ND virus. At the same time, PCR analysis confirmed the presence of the ND virus in 11 out of the 12 cases, with strains showing high genetic similarity to local field strains but notable variations from existing vaccine strains. The analysis indicated that the strains belonged to the avian paramyxovirus 1 lineage, with a sequence identity of 99.63% among them. The study highlights the need for ongoing molecular surveillance of ND virus in Nigeria to guide vaccination strategies considering the genetic diversity of circulating strains.

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INTRODUCTION

Poultry farming is an important component of Nigeria's agrarian economy, ensuring the available levels of food security and hence employment and economic stability **(Oloso *et al.*, 2020; Okwuokenye *et al.*, 2024).** It brings income to many homes, besides providing essential protein from chicken meat and eggs **(Oloso *et al.*, 2020).** However, this critical industry is severely impacted by Newcastle Disease (ND), a highly contagious viral infection caused by avian *paramyxovirus type 1* (APMV-1), which belongs to the *Paramyxoviridae* family **(Megahed *et al.*, 2020).** Newcastle Disease is characterized by a wide range of clinical manifestations, from mild respiratory signs to severe systemic disease with high mortality rates, depending on the viral strain and host factors **(Japhet, 2022).** In Nigeria, ND remains endemic and accounts for over 40% of poultry disease cases reported in some regions, posing severe economic consequences for smallholder farmers and commercial producers **(Muhammed *et al.*, 2020; Amoia *et al.*, 2024).**

The prevalence of ND in Nigeria varies across regions, with rates as high as 42.6% reported at the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria **(Muhammed *et al.*, 2020).** In Ilorin, a seroprevalence of 13.25% was documented among indigenous chickens **(Olorunshola *et al.*, 2022)** while in Kaduna, rates have reached 31% **(Adanu *et al.*,**

2021). These variations highlight regional differences in disease burden and emphasize the impact of biosecurity practices, vaccination coverage, and the role of live bird markets in virus transmission. Live bird markets, in particular, serve as significant reservoirs for the ND virus (NDV), enabling its spread and reintroduction into commercial and backyard poultry systems **(Chinyere *et al.*, 2024).**

The genetic diversity of NDV strains circulating in Nigeria further complicates the control of the disease. In Nigeria, significant genotypic diversity is evident, with class II genotypes VII and XIV being the most frequently reported **(Sajo *et al.*, 2022; Chinyere *et al.*, 2024).** This diversity poses challenges for vaccine efficacy since mismatches between vaccine strains and circulating field strains have been documented **(Bello *et al.*, 2018; Adanu *et al.*, 2021; Sajo *et al.*, 2022).** The persistence of ND outbreaks in vaccinated flocks, despite all the vaccination campaigns, calls for molecular surveillance and locally tailored vaccine development **(*Bello et al.*, 2018).**

Given the importance of this sector in Ibadan, which is one of the major poultry-producing centers in Nigeria, addressing the challenges posed by NDV genetic diversity is paramount. Several studies **(Ei *et al.*, 2017; Unigwe *et al.*, 2020)** have been carried out on the seroprevalence of this disease in this locality, but molecular studies on strains of NDV are limited in Ibadan.

The study was therefore carried out to investigate the prevalence of ND, characterize the genetic diversity of circulating strains, and assess their compatibility with the vaccine strains being used in Nigeria. The obtained findings would be highly valuable in guiding suitable vaccination strategies, ensuring improved ND control practices, and enhancing the resilience of the poultry sector in Nigeria.

MATERIALS AND METHODS

Study Area

The present study was carried out in Ibadan, the capital of Oyo State, lying between latitude 7° 23′ N and longitude 3° 56′ E, in Southwest Nigeria. Geographically, it lies within the transition zone of the forest-savanna zone, about 128 km northeast of Lagos **(Aikiraji et al., 2016).** It is one of the largest cities in Nigeria by both population and land area. The area serves as the central hub of the country's poultry industry, and Ibadan is a major distribution point for all types of poultry products, including day-old chicks, broilers, and point-of-lay pullets, alongside essential poultry inputs such as drugs, vaccines, and feed ingredients. There are many live bird markets within the city, including the prominent Shasha market that acts as the trading center for different bird species. Most of these birds are supplied by small-scale farmers from surrounding rural areas and traders from northern Nigeria, who additionally transport cattle, sheep, and goats to the southwest region **(Oluwole *et al.*, 2012).**

Ethical Approval

This study was conducted in compliance with ethical standards for research involving animals. Approval was obtained from the University of Ibadan Animal Care and Use Research Ethics Committee under permit number UI-ACUREC/077-0524/22. All procedures followed institutional and international guidelines for the humane treatment of animals in research. Only carcasses submitted for diagnostic evaluation were used ensuring that no additional harm was caused to live animals. Measures were taken to handle and dispose of carcasses appropriately, ensuring biosecurity and ethical research practices throughout the study.

Study Design

A cross-sectional design was used in this study to investigate the occurrence and molecular characteristics of Newcastle disease virus strains in chicken flocks within Ibadan, Nigeria. Data were collected over four months (June to September 2024), from dead chickens that had been submitted for diagnostic evaluation at CHI Diagnostic Laboratory at Oluyole Estate, Ibadan, and the Poultry Diseases Clinic at the University of Ibadan's Veterinary Teaching Hospital.

Necropsy and Sample Collection

Following external examination, chicken carcasses submitted for post-mortem analysis were disinfected by immersion in a disinfectant solution. A systematic necropsy was conducted, and gross pathological lesions were recorded. Carcasses showing Newcastle Disease lesions such as haemorrhages in the small intestine, proventriculus, caecal tonsils, and colon, along with tracheal congestion (with or without mucoid exudate), were selected for sampling. Tracheal and cloacal swabs were aseptically collected using sterile swabs during the necropsy procedure. The swabs were placed in Virus Transport Medium (VTM) for viral preservation, properly labelled, and stored at -10°C for subsequent analyses. Swabs from each farm were pooled by sampling point, yielding one tracheal sample and one cloacal sample per farm. A total of 106 farms were included in the current study.

Haemagglutination and Haemagglutination Inhibition Tests

The haemagglutination (HA) and haemagglutination inhibition (HI) tests were conducted as described by FAO (2023) with slight modifications for the detection and confirmation of ND virus. For the HA test, fifty microliters of each pooled sample were transferred into wells of a U-bottomed microtiter plate (in duplicates), including positive controls containing

diluted LaSota vaccine virus (1HAU) and negative controls containing normal saline. An equal volume (50 µl) of 0.5% washed chicken red blood cells was added to each well. The plate was gently mixed using a microplate shaker and incubated at room temperature (27°C) for 30 minutes. Haemagglutination activity was determined by visual comparison of the sample wells with the control wells. The clumping of red blood cells in the sample wells indicated a positive result. Positive HA samples were selected for further testing by the HI test.

The HI test was used to confirm the presence of ND virus and its inhibition by NDV-specific antibodies obtained from a vaccinated flock. Fifty microliters of HA-positive samples were dispensed into wells of a U-bottomed microtiter plate, alongside positive and negative controls. Fifty microliters of NDV-specific antiserum were added to each well and mixed thoroughly. After 15 minutes of incubation at room temperature, 50 µl of 0.5% washed chicken red blood cells was added, mixed, and incubated for an additional 30 minutes. Results were read visually by comparing the sample wells to the control wells. Inhibition of haemagglutination was recorded as a positive result when red blood cells settled as a clear button at the bottom of the wells. Samples with positive HI results were stored for subsequent molecular analysis.

Reverse Transcription - Polymerase Chain Reaction (RT-PCR)

RNA was extracted using the QIAGEN kit according to the standard protocols. The samples were lysed with AVL buffer containing carrier RNA, vortexed, and incubated at room temperature to ensure complete lysis. Ethanol was added to the lysed samples, and they were passed through spin. After washing with AW1 and AW2 buffers through columns, high-speed centrifugation was performed. Then, RNA was eluted with AVE buffer and stored at -80°C until further use.

The conserved fusion protein gene of ND virus was targeted for primer designing by using sequences from the NCBI database. These primers were synthesized by Inqaba Biotechnical Industries (South Africa) and then verified for specificity and alignment with conserved regions of ND virus. RT-PCR was performed in the SYBR Green master mix containing NDV-specific primers (forward: GCATTGCTGCAACCAATGAAG, reverse: ATCCATATTGCCACCAGCTAAA), Taq DNA polymerase, and reverse transcriptase in a one-step reaction. Reverse transcription was allowed to proceed at 42°C for 30 minutes and was followed by an initial denaturation at 94°C for 5 minutes. Amplification was carried out for 30 cycles, with the following steps: denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 1 minute. A final extension step was carried out at 72°C for 10 minutes. The integrity of the amplified 270 bp products was checked by electrophoresis on a 1.5% agarose gel stained with ethidium bromide and visualized under UV light **(Sambrook and Russell, 2006).** The amplified fragments were purified by ethanol precipitation and the concentration of each was determined by a Nanodrop spectrophotometer. The purified DNA was then stored at -20°C until subsequent sequencing.

Sequencing

Sequencing was performed following the protocol provided by the manufacturer (Applied Biosystems) using the Genetic Analyzer 3130xl, USA, along with the BigDye Terminator v3.1 Cycle Sequencing Kit from Thermo Fisher Scientific, USA. Sequence editing and cluster alignment were carried out using BioEdit software, while genetic analysis was conducted using MEGA 6.

Data Analysis

Descriptive statistics were used to assess the occurrence of ND in Ibadan chicken flocks. All the results, including the occurrence rates and genetic comparisons, were presented in the text for clarity. Genetic analysis was done using MEGA 6. Multiple Sequence Alignment (MSA) was performed with ClustalW, as implemented in the MEGA 6 program package, for identifying nucleotide variations and conserved regions. Phylogenetic trees were generated using the ML approach with 1,000 bootstrap replicates to make sure the branching patterns were resilient. Isolates from ND virus Ibadan were compared to reference strains such as Lasota (KU665480.1), Lasota (KU665481.1), and B1 (M24708.1) and local Nigerian strains such as MN339529.1, MN339527.1, and 0K072692.1. Comparisons were also made with field strains from other regions, including China, Egypt, Pakistan, and the United States.

RESULTS

Necropsy examination of some cases revealed haemorrhages in the intestine, proventriculus, and colon, as well as congested tracheae with or without mucoid exudate. A total of 297 poultry cases were submitted during the study period, with 281 cases to the Chi Farm Diagnostic Laboratory and 16 cases to the Poultry Disease Clinic at the Veterinary Teaching Hospital, University of Ibadan. Based on the observed pathological lesions, 26 cases were suspected to be Newcastle Disease (ND), yielding an occurrence rate of 8.8%. All 26 suspected cases were tested using the haemagglutination (HA) test, and haemagglutination activity was confirmed in all samples (100%) **.** Further analysis was performed using the haemagglutination inhibition (HI) test with known ND-positive antiserum, which identified 12 positive samples out of the 26 HA-positive cases, representing 4.04% of the 297 poultry cases examined **(Table 1).**

Table 1: Immunological Tests Results for Newcastle Disease in the suspected Poultry Cases

	Total Examined Cases	Positive Results	Negative Results	Overall Percentage Positive cases (%)
Haemagglutination (HA)	26	26 (100%)	0(00%)	8.8%
Haemagglutination Inhibition (HI)	26	12(46.15%)	14(53.83%)	4.04%

All the (12) samples were analysed using PCR showed bands at a molecular weight of approximately 270 bp, consistent with the F-gene of Newcastle Disease Virus (NDV), except for sample number 8, which tested negative **(Fig. 1).** This confirmed that 11 out of the 297 poultry cases (3.7%) tested positive for Newcastle Disease Virus (NVD) using (PCR) **(Fig. 1)** analysis revealed that all the samples, except one (sample number 8), showed bands consistent with the F-gene of the Newcastle disease virus, confirming infection in 11 out of the 297 cases, which equates to a diagnostic rate of 3.7%.

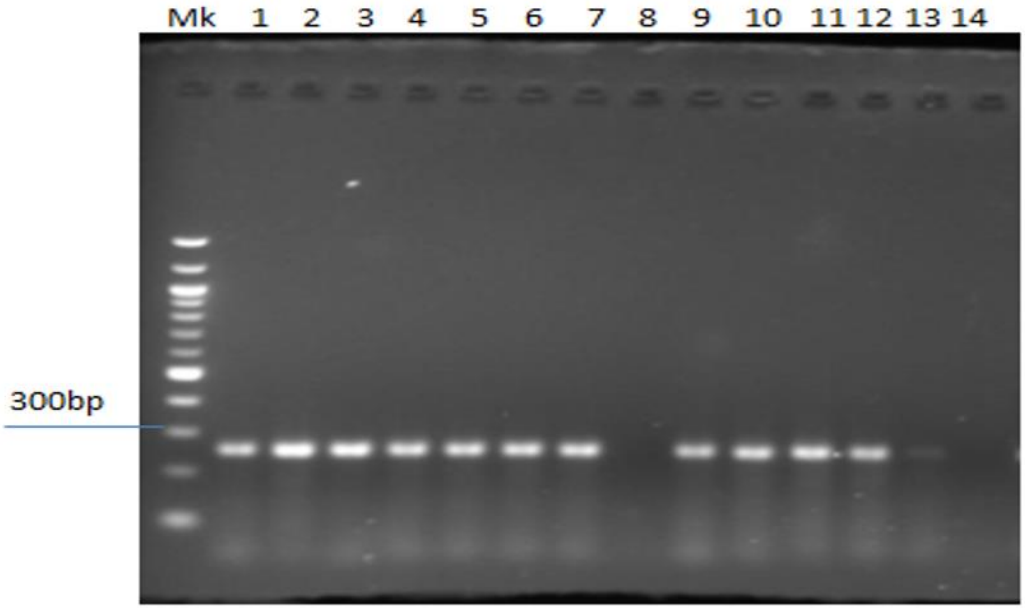

Fig.1: Agarose gel electrophoresis (*1.5% agarose gel*) showing the positive amplification of the PCR products of fusion gene from selected nucleic acid extracted from chickens diagnosed with Newcastle disease. Expected band size of molecular weight of approximately 270bp.

Analysis of the "F" gene isolates (F4T, F11T, F13T, F15T, F16T, F18T, F19T, F22T, F25T, and F26T) using NCBI BLAST confirmed them as Avian paramyxovirus 1, the virus responsible for ND. These strains showed a perfect match with the reference sequence, achieving the highest alignment score of 497 497bp, complete query coverage, and an extremely low E-value of 2.00E-136. Their high sequence identity of 99.63% reflects remarkable genetic similarity, indicating that they likely originate from the same viral lineage or outbreak.

The multiple alignment of the eight NDV strains from eight samples (NDV1, NDV3, NDV4, NDV5, NDV6, NDV7, NDV8 and NDV9 Chicken Ibadan) with reference strains from across the world revealed several nucleotide substitutions, especially when compared to reference strains such as the Lasota vaccine strain (U22292.1, KU665480.1, KU665481.1) and B1 vaccine strain (M24708.1). All Ibadan strains shared a high degree of sequence similarities with Nigerian field strains (MN339529.1, MN339527.1, and 0K072692.1), but genetic variations were observed in specific regions of the genome **(Fig. 2).** Among the Ibadan strains, NDV 8 Chicken Ibadan was the closest to the B1 vaccine strain, sharing conserved nucleotide sequences at positions 105-140 with fewer substitutions compared to other Ibadan isolates. Although the other isolates (NDV 1-7) had substitutions such as G→T and C→A at positions 120-140, these were absent in NDV 8, which retained alignment with B1 in these regions. NDV 8 also exhibited fewer mutations at positions 160-180 with only C→T and G→A substitutions, compared to additional variations observed in other Ibadan isolates.

Compared to the Lasota vaccine strain, all Ibadan isolates, including NDV 8, had substitutions at positions 110-140, including G→A, A→G, and T→C, as well as C→T and G→A substitutions at positions 160-180. However, NDV 8 was more closely related to B1 than Lasota, especially at positions 120-140, where NDV 8 retained conserved sequences that were altered in the other isolates (NDV 1-7). There are constant mutations at positions 190-210, including A→T and C→G, in all of the Ibadan isolates. These distinctly separated them further from B1. Generally, NDV 8 was less mutated compared to other Ibadan isolates, which was reiterative of its closeness to B1 **(Fig. 2).**

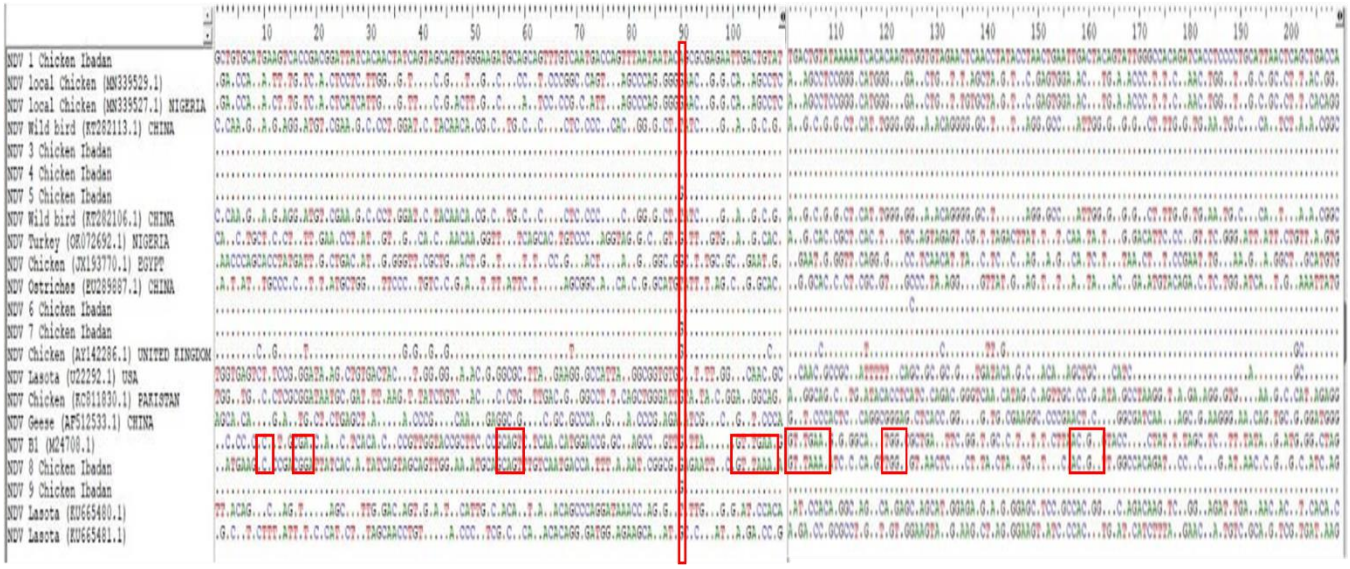

Fig. 2: Amino Acid Alignment of Newcastle Disease Virus (NDV) strains from Poultry Cases in Ibadan, Nigeria (2024), Showing Conservation and Polymorphisms Across Key Regions.

Phylogenetic analysis revealed that the Ibadan NDV strains (NDV 1-9) were grouped into a distinct monophyletic cluster, with the highest genetic similarity observed to Nigerian NDV strains (MN339529.1, MN339527.1) **(Fig. 3)**. Unlike the other Ibadan strains, NDV 8 exhibited a longer branch length from the main Ibadan cluster, indicating greater genetic divergence within the group **(Fig. 3)**. Regional strains from Egypt (JX193770.1), Pakistan (KC811830.1), and Turkey (OK072692.1) exhibited moderate relatedness, while greater phylogenetic distances were noted for global strains from the UK (AY142286.1), USA (U22292.1), and China (KT282113.1, KT282106.1, AF512533.1).

A separate clade was formed by vaccine strains, including NDV Lasota (U22292.1, KU665481.1) and NDV B1 (M24708.1), which remained distinct from the Ibadan strains. However, NDV 8 (Ibadan) was positioned outside the main Ibadan cluster, displaying greater phylogenetic proximity to NDV B1 **(Fig. 3)**. A shorter branch length was observed between NDV 8 and NDV B1 compared to other Ibadan isolates. While remaining part of the broader Ibadan lineage, NDV 8 showed lower genetic similarity to the other Ibadan strains and was placed in closer association with NDV B1 within the phylogenetic tree.

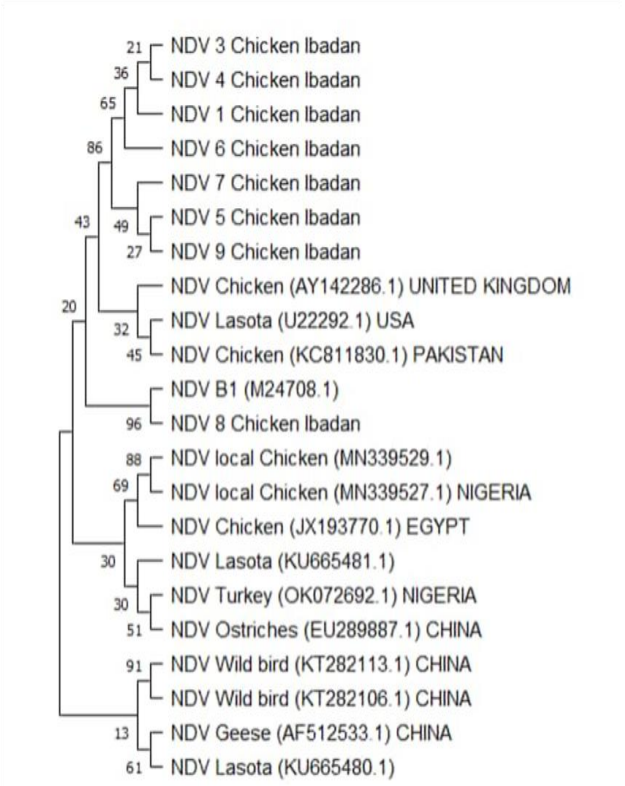

Fig. 3: Phylogenetic Tree of Newcastle Disease Virus (NDV) Isolates from Ibadan, Nigeria (2024), and Their Evolutionary Relationships with Global Strain.

DISCUSSION

This study was carried out to investigate the occurrence of Newcastle Disease (ND) in chicken flocks within Ibadan, characterize the genetic diversity of the circulating NDV strains, and assess their compatibility with the vaccine strains used in Nigeria.

In suspected ND cases, post-mortem examination showed intestinal, proventricular, and colonic haemorrhages and congested tracheae, some with mucoid exudate. The described lesions agree with those caused by virulent strains of NDV, particularly viscerotropic and neurotropic forms, which cause vascular damage with mucosal inflammation **(Mariappan *et al.*, 2018; Igwe, 2024).** The 8.8% occurrence rate based on gross pathology suggests a notable disease burden; however, necropsy alone is not conclusive, as similar lesions may also be caused by other avian diseases such as highly pathogenic avian influenza (HPAI) and infectious bronchitis virus (IBV) **(Broomand *et al.*, 2018; Moharam *et al.*, 2019).**

The HA test showed that all the 26 suspected cases harboured haemagglutinating activity, indicating the existence of a virus with haemagglutination properties; however, HA is non-specific and can detect other avian paramyxoviruses, such as avian influenza virus (AIV) and avian metapneumoviruses **(Suarez *et al.*, 2020).** The HI test confirmed NDV in only 12 cases, and this resulted in a positivity rate of 4.04% out of the 297 poultry cases put into examination. This discrepancy would, therefore, suggest a possible high number of false positives either due to cross-reactivity with other viruses or antigenic variation leading to false negatives, particularly in vaccinated flocks **(Yang *et al.*, 2017; Mahmood *et al.*, 2024).** Other factors contributing to such discrepancies may include sample handling, virus titration, and specificity of antisera from other groups. The low confirmation rate of NDV highlights the limitation of serological assays and the need for molecular diagnosis, such as RT-PCR and sequencing methods, to improve diagnostic accuracy and differentiation of NDV from other haemagglutinating avian pathogens **(Miller and Torchetti, 2014; Ashraf *et al.*, 2016).**

The findings of this study indicate that the prevalence of ND in Ibadan is lower than in other Nigerian regions, as previous studies have reported rates of 42.6% in Zaria **(Muhammed *et al.*, 2020),** 13.25% in Ilorin **(Olorunshola *et al.*, 2022)** and 31% in Kaduna **(Adanu *et al.*, 2021).** This suggests that while ND is considered endemic in Nigeria, its occurrence may vary significantly by location, possibly due to differences in biosecurity measures, vaccination coverage, or poultry management practices.

Polymerase Chain Reaction (PCR) analysis confirmed NDV in 11 out of 297 poultry cases (3.7%), with all positive samples exhibiting the expected molecular weight of approximately a 270 bp band corresponding to the F-gene. The lower PCR-confirmed prevalence compared to the 8.8% occurrence based on gross pathology highlights the limitations of lesion-based diagnosis, which may overestimate ND cases due to similarities with other avian diseases such as avian influenza. The discrepancy between serological and molecular results suggests potential false positives in HA due to cross-reactivity with other hemagglutinating viruses or false negatives in HI due to antigenic variation or vaccination effects. The 3.7% PCR detection rate observed in this study is considerably lower than the prevalence rates reported in other Nigerian regions, such as 15-30% in commercial poultry flocks and 42.6% in some locations **(Bello *et al.*, 2018).** This variation may be due to the difference in NDV strain circulating, vaccination coverage, biosecurity, and poultry management practices. Some other methodological differences such as sample type, diagnostic approach, and severity of outbreak during sampling could also account for the discrepancy observed.

Genetic characterization of the "F" gene confirmed them as Avian paramyxovirus 1, with a high sequence identity (99.63%) to reference NDV strains, suggesting circulation of a conserved viral lineage. The high alignment score (497) and extremely low E-value (2.00E-136) suggest that these isolates are highly conserved and likely originated from a single lineage or outbreak. This genetic stability aligns with findings from Bello et al. (2018), who reported limited genomic variation among certain NDV strains in Nigeria despite ongoing viral evolution. However, the high sequence similarity raises concerns regarding persistent NDV transmission despite vaccination, possibly due to vaccine strain mismatch or antigenic drift. However, MSA revealed nucleotide substitutions, particularly when compared to vaccine strains such as La Sota and B1. The observed mutations indicate ongoing viral evolution, which may affect vaccine efficacy, host adaptation, and immune evasion. This finding is consistent with previous studies suggesting that genetic divergence between vaccine strains and circulating field strains poses a major challenge for ND control in Nigeria, as genotype mismatches may reduce vaccine-induced protection **(Bello *et al.*, 2018; Sajo *et al.*, 2023).** Likewise, **Sajo *et al.*, (2022)** reported that, despite widespread NDV vaccination, outbreaks persist due to antigenic drift and the genetic variability of circulating strains.

Phylogenetic analysis further highlighted the evolutionary relationships among the Ibadan strains. NDV1, NDV3, NDV4, NDV6, NDV7, and NDV9

clustered closely with Nigerian NDV strains (MN339529.1, MN339527.1) and NDV strains from the United Kingdom and Pakistan and the Lasota vaccine strain from the United States, while NDV8 followed a distinct trajectory, aligning more closely with the B1 vaccine strain. This suggests that NDV8 has undergone fewer mutations and may retain greater antigenic similarity to B1-based vaccines. However, the presence of mutations in all Ibadan strains indicates ongoing adaptation, which could influence viral transmissibility and pathogenicity. The identified genetic differences suggest that currently used vaccines may not provide optimal protection against local NDV strains. This finding corroborates earlier research by **Shahar *et al.*, (2018)** which demonstrated reduced vaccine efficacy in regions characterized by high genetic diversity of NDV.

The observed genetic relationships with strains from Egypt, wild birds, turkeys, and ostriches highlight the role of interspecies transmission in NDV evolution, further complicating control strategies. Similar findings were reported in Angola, where NDV isolates showed genetic links to strains from neighboring countries, reinforcing the significance of transboundary viral movement in Africa **(Henriques *et al.*, 2023).** The persistence of virulent NDV genotypes despite vaccination, as documented in various African studies, underscores the need for continuous genomic monitoring, improved vaccine formulations, and enhanced biosecurity measures to mitigate NDV transmission **(Bello *et al.*, 2018; Henriques *et al.*, 2023; Sajo *et al.*, 2023).** These findings highlight the importance of targeted vaccination strategies using genotype-matched vaccines and real-time surveillance to prevent outbreaks and ensure effective ND control in Ibadan.

Limits of the study

The sampling approach was based on the carcasses submitted to CHI Farms Diagnostic Laboratory and the Poultry Diseases Clinic at the University of Ibadan's Veterinary Teaching Hospital only, as such, cases that were not reported to these diagnostic points during the period may not have been included. This could lead to an underestimation of ND occurrence.

CONCLUSION

The present study provided valuable insight into the occurrence and molecular characteristics of ND virus in chicken flocks in Ibadan. The results showed a lower-than-expected occurrence of ND in Ibadan. The phylogenetic analysis showed that ND virus isolates from Ibadan form a distinct genetic cluster, closely related to other local strains but genetically divergent from vaccine strains and global strains. These findings, therefore, support the need for increased molecular surveillance of strain evolution and emerging variants of the virus. In addition, good biosecurity measures and proper awareness among poultry farmers regarding disease management will assist in controlling ND outbreaks and thus ensuring sustainability in the poultry industry in Nigeria.

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Conflicts of Interest

The authors declare no conflict of interest. The sponsors had no role in the design, execution, interpretation, or writing of the study.

Authors' contributions

Oladele, O.A. conceptualized and designed the research; Balde, A. conducted the study, analysed data and wrote the initial manuscript; Bakre, A. A. contributed to study design and data analysis; Onidje, E. contributed to interpretation and manuscript writing; Eyarefe, O.D. and Igado O.O. contributed to manuscript revision. All authors approved the final version.

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