Phylogenetic analysis of 2015-2016 Highly Pathogenic Avian Influenza (HPAI) H5N1 viruses, Nigeria

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INTRODUCTION

Avian influenza (AI) is a disease of agro-economic and public health importance which generally manifests as respiratory infection of birds (Li *et al.*, 2004). The severe form known as highly pathogenic AI (HPAI) affects virtually every organ and tissue resulting in excessively high mortalities, as well as causing sporadic human cases where close contact with infected poultry occurs. Influenza A viruses are classified into subtypes on the basis of the antigenic combination of their surface glycoproteins haemagglutinin (HA) and neuraminidase (NA) (Munster & Fouchier, 2009). All naturally occurring HPAI viruses isolated to date have been of the H5 and H7 subtypes, although most H5 and H7 viruses are of low pathogenicity (OIE, 2014).

HPAI, characterized by sudden onset and excessively high mortality, was first reported in Nigeria in 2006 and again in 2015 with devastating economic consequences. Here, the complete genomes of 100 isolates collected between 2015 and 2016, covering 16 states and the Federal Capital Territory, were analyzed

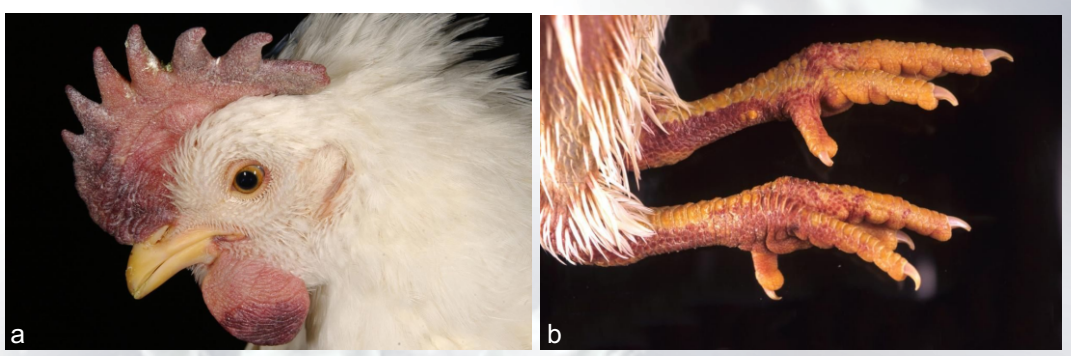

Figure 1a - c: Chicken infected with HPAI showing cyanosis of combs and wattles (a). Haemorrhages of the shank of chicken infected with HPAI (b). Massive mortalities associated with HPAI in poultry (c).

AIMS

To provide a better understanding of the genetic characteristics and evolution of the H5N1 viruses currently circulating in Nigeria, and the spatiotemporal relationship with other viruses globally, we carried out whole genome sequencing and phylogenetic analyses.

MATERIALS AND METHODS

Sample collection: Samples from sick/dead birds consistent with case definition of HPAI from field outbreaks were collected from December 2014 until June 2016 (Fig 2).

Virus Isolation and Amplification: Virus isolation was conducted in embryonated chicken eggs (OIE, 2015), infected allantoic fluids were harvested and viral RNAs were obtained to amplify complete influenza A virus genome using SuperScript III one-step reverse transcription-PCR (RT-PCR) and protocol described by Zhou *et al*. (2009). Amplified products were visualized on a 0.7% agarose gel stained with GelRed.

Whole genome sequencing: Using Nextera DNA XT sample preparation kit (Illumina), DNA libraries were obtained, indexed libraries were pooled in equimolar concentrations and sequenced in multiplex analysis for 300-bp paired-end on Illumina MiSeq according to the manufacturer's instructions.

Data analysis and phylogeny: Consensus sequences were obtained and high quality reads were aligned against a reference genome using BWA v0.7.12. A BLAST search for each gene segment of 100 HPAI H5N1 viruses was performed in the GISAID database and the most closely-related sequences were downloaded, as well as sequences of H5N1 viruses from the 2006-8 outbreaks in Nigeria. Sequence alignments were performed using the on-line program Mafft v.7.0. Maximum likelihood (ML) phylogenetic trees were generated in PhyML v.3.1

RESULTS AND DISCUSSION

The complete genomes of 100 H5N1 viruses collected from January 2015 to June 2016 in Nigerian poultry were sequenced and submitted to the GenBank database under accession numbers MF112254-MF113041. The deduced amino acid sequence (PQRERRRK_R*GLF) at the HA cleavage sites is characteristic of an HPAI virus strain and possesses an amino acid deletion at position 329 compared to clade 2.2 viruses previously reported in Nigeria. The topology of the phylogenetic tree of the HA gene demonstrated that all the viruses analysed fall within clade 2.3.2.1c and cluster with H5N1 viruses identified since 2014 in Asia, the Middle East, East Europe and other West African countries including Niger, Ghana, Burkina Faso and Ivory Coast. Two previously defined subgroups within this clade, WA1 and WA2 were identified. Specific amino acid signatures were considered in defining sub-groups observed within this clade (Fig 3). The Nigerian viruses collected in 2015 are dispersed throughout the tree, indicating the possible occurrence of multiple independent introductions or the evolution of the virus into multiple genetic groups (Fig 3)

Similarly, topologies of the phylogenies inferred for the remaining gene segments indicate that the Nigerian viruses cluster with viruses of 2.3.2.1c clade collected in African, Middle Eastern and Asian countries since 2014 with characteristic amino acid signatures observed in all the gene segments. There is evidence of intra-clade reassortment.

CONCLUSIONS

An epidemiological linkage of HPAI H5N1 clade 2.3.2.1c introduction from Eastern Europe to Nigeria through migratory waterfowls has been reinforced by our findings in this study. This, however, does not preclude the role of poultry/poultry products trade between Nigeria and these countries. Further study, especially within the migratory birds, may be required to ascertain their role in the introduction of the virus. This revelation underscores the need to up biosecurity measures and surveillance activities for effective control of HPAI and to slow down the rate of evolution of the virus in Nigeria

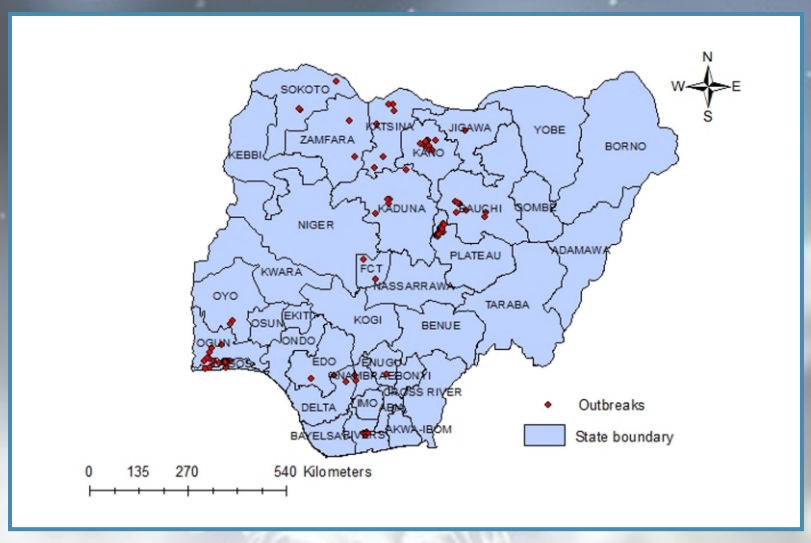

Figure 2: Map of Nigeria showing locations of H5N1 viruses analysed in this study

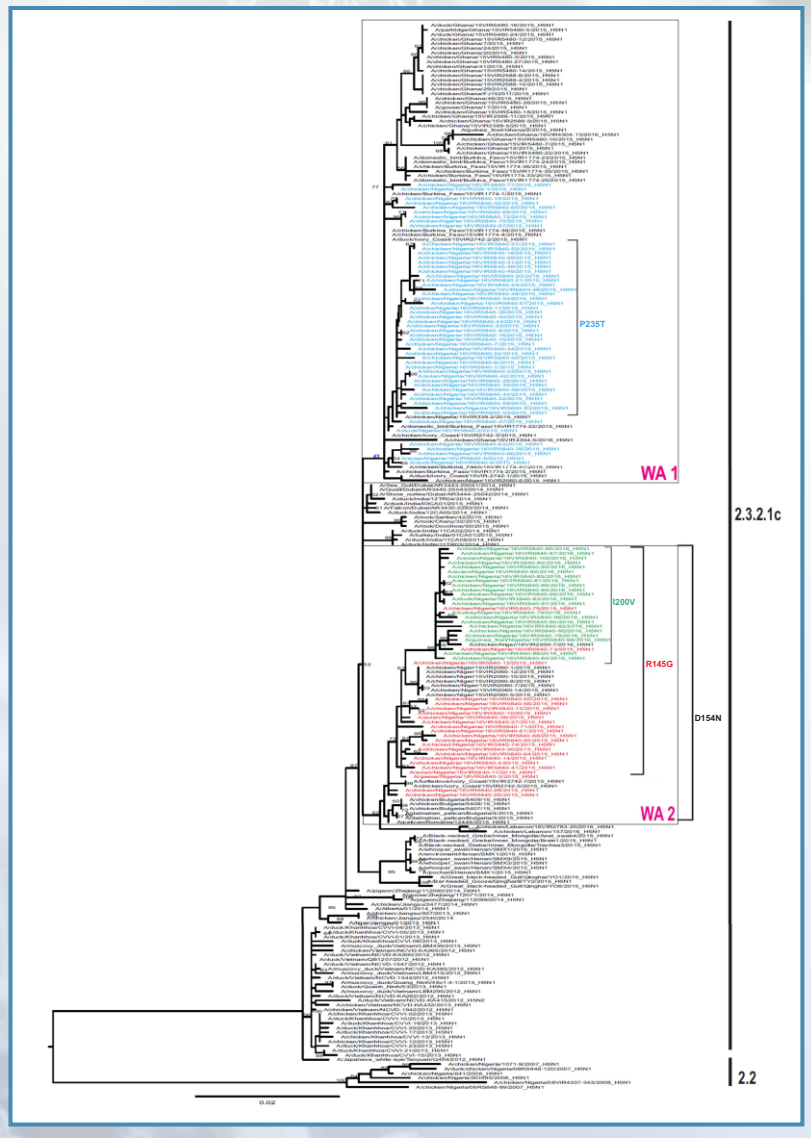

Figure 3: Maximum-likelihood phylogenetic tree of the HA gene segment of highly pathogenic avian influenza (H5N1) viruses from Nigeria. All the 2016 viruses (except one) cluster within the green group with good bootstrap value, 620 (62%). This group also clusters with some 2015 viruses (red) viruses, which appear to be the progenitor. While the remaining 2015 viruses (blue) cluster separately

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