The genetic and antigenic diversities of H9N2 avian influenza viruses infecting poultry in Bangladesh

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Introduction

H9N2 avian influenza viruses (AIVs) are endemic and persistently circulate within Bangladeshi poultry populations, causing substantial production losses of eggs and meat. Vaccination is crucial for inducing protective immunity and mitigating disease impacts.

However, the genetic and antigenic dynamics of contemporary H9N2 AIVs in Bangladeshi poultry are poorly understood, hindering accurate antigenic matching of vaccine seed strains to the circulating viruses.

Methods

1. Sampling of Bangladeshi poultry markets and whole genome sequencing of AIV positive isolates.
2. Phylogenetic characterisation of haemagglutinin (HA).
3. Investigation of antigenic disparity between virus groups/clusters by reverse genetic (RG) generation of representative viruses.
4. Investigation and identification of antigenic residues by single-site mutagenesis and haemagglutination inhibition assay (HI).

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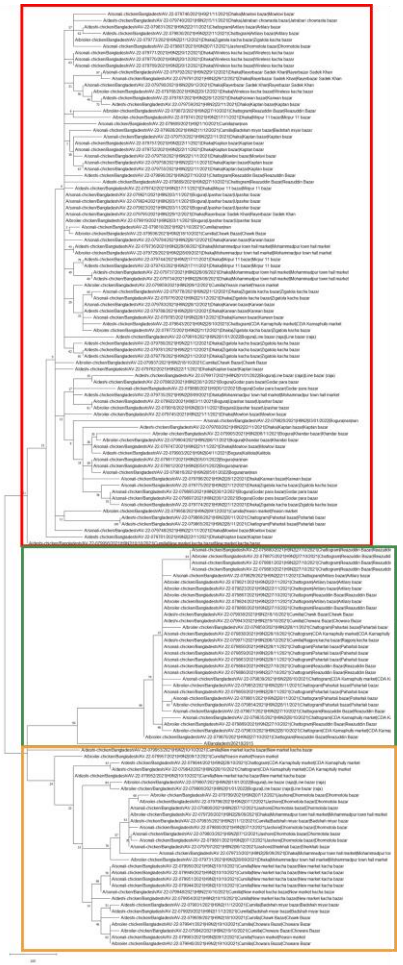
Results



H9N2 HA sequences isolated from Bangladeshi poultry markets (2021/2022) clustered into three (red, green and orange) subgroups of the G1 lineage, characterised by distinct conserved residues.

***Figure 1**: Maximum likelihood phylogenetic tree of H9N2 HA sequences. Three genetically distinct branches are coloured red, green and orange.*

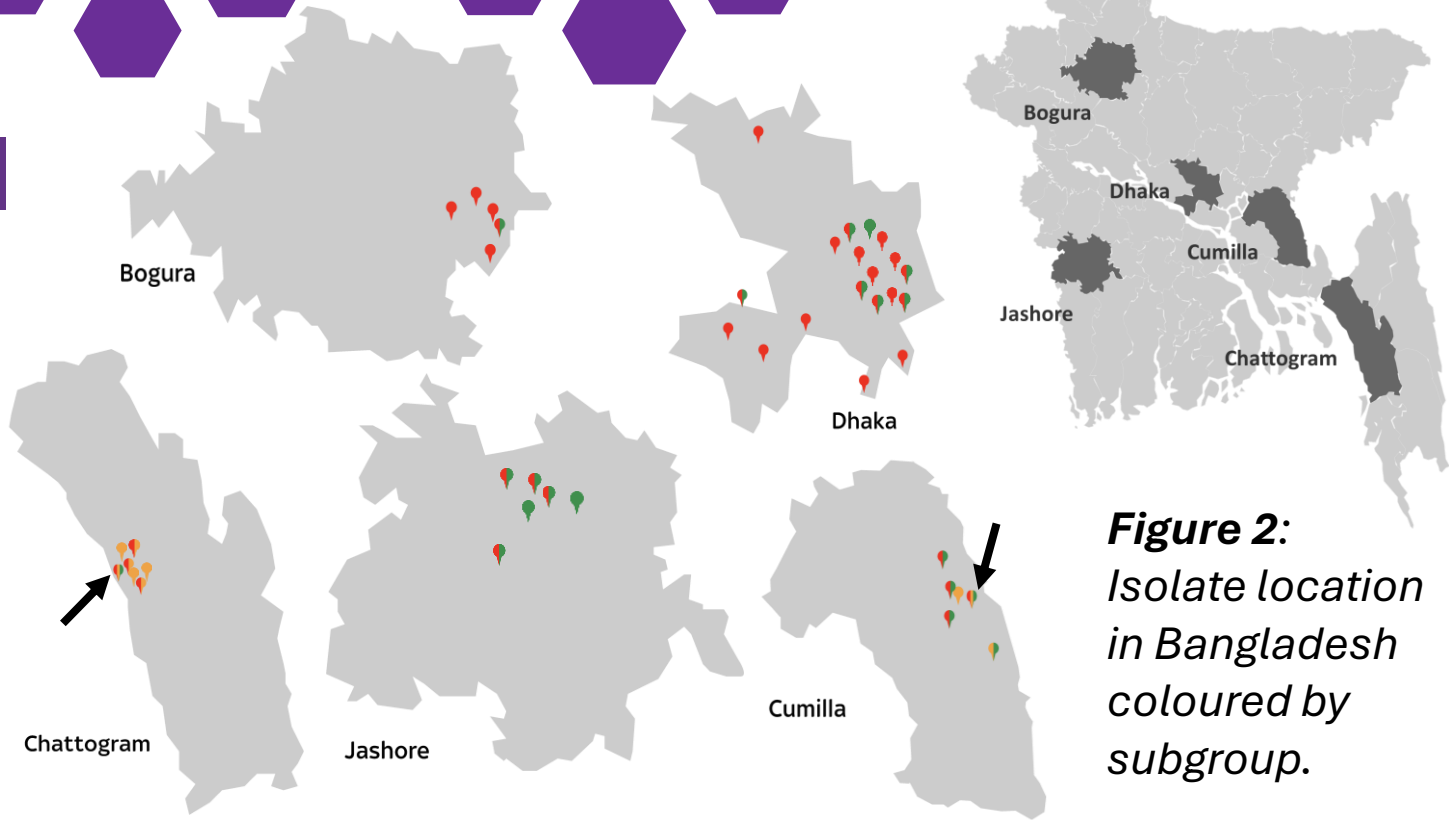


***Figure 2**: Isolate location in Bangladesh coloured by subgroup.*

The location of each isolate was plotted to identify the distribution of each subgroup across sampled regions.

- Red and green subgroups isolated in all regions.
- Orange subgroup localised to Cumilla and Chattogram.
- Two sites isolated all three subgroups simultaneously (indicated by arrows).

Results Continued

The residues conserved within each subgroup were integrated into a representative HA by site-directed mutagenesis to investigate their antigenic influence, using homologous sera in haemagglutination inhibition assays.

Two novel mutations were identified with HI titre changes >2log2, with the first conserved in the green subgroup and the other in the orange.

Conclusions

Bangladeshi H9N2 AIVs have diversified into three genetically distinct subgroups, co-circulating and simultaneously isolated from poultry markets. Some conserved residues differing between the groups are significantly antigenically diverse.

Consequently, antigenic matching of vaccines to field strains would require multiple antigens to incorporate the epitope disparity between the subgroups.

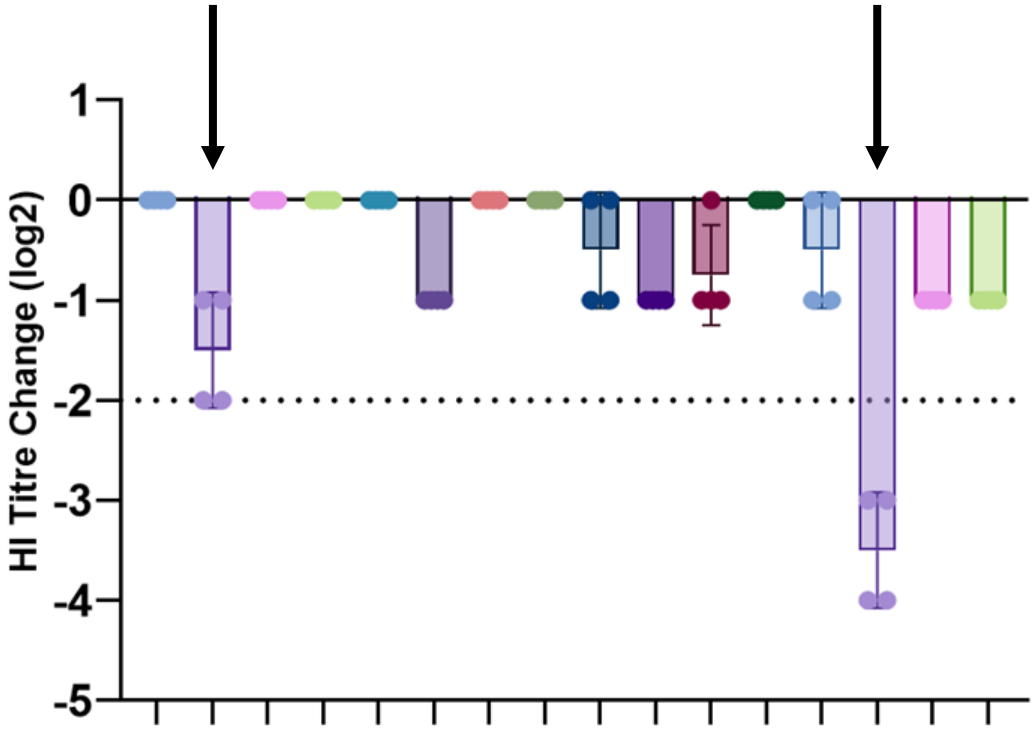


***Figure 3**: HI titre change of residue mutations to homologous sera. Titre changes >2log2 are deemed significant (indicated by arrows).*