Mapping antigenic diversity to investigate putative antigenic residues of H5 avian influenza viruses

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Introduction

H5Nx avian influenza viruses (AIVs) continue to circulate within and between avian populations, with devastating socio-economic consequences and posing a threat to public health. The haemagglutinin (HA) has diverged into >30 genetically and antigenically distinct clades and subclades, including the epizootic clade 2.3.4.4b. This diversity challenges protective efficacy of poultry vaccines with poor antigenic matching of vaccines to field strains. Therefore, methods to improve vaccine seed selection based upon antigenic data were generated to enhance protection of poultry.

Methods

1. Generation of clade representative viruses by reverse genetics (RG) (n=22).
2. Raising of antisera in chickens to measure antibody titre and antigenic cross-reactivity of RG viruses by haemagglutination inhibition assay (HI).
3. Development of methods to predict antigenic residues that drive antigenic change using pairwise comparison of antigenic and genetic diversities.
4. Integration of residues by single-site mutagenesis (SDM), with antigenicity confirmed by HI.

Results

To characterise the antigenic diversity of H5 clades, 22 viruses were generated from clades 1 to 2.3.4.4h using RG techniques.

Sera was raised to each virus in white leghorn chickens and serologically characterised by HI. The cross-reactivity of HI titres were visualised by antigenic cartography (Figure 1).

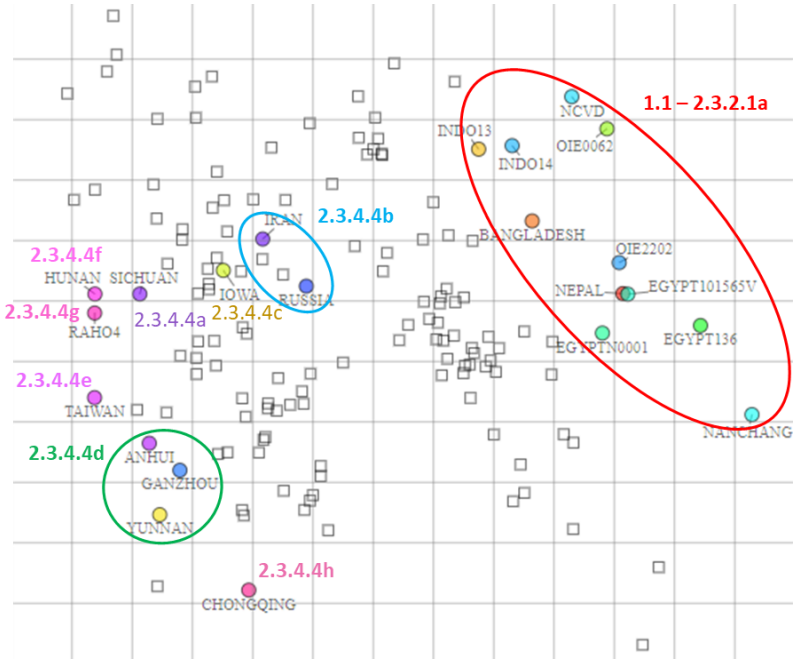

***Figure 1**: Antigenic cartography of homologous and heterologous H5 antisera cross-reactivity by HI. 1 square = 1 antigenic unit = 2log HI titre.*

To predict residues driving antigenic diversity, antigenic distances between virus plots were plotted against their HA amino acid disparity (Figure 2); plots with the greatest antigenic to genetic ratio were investigated by SDM/HI.

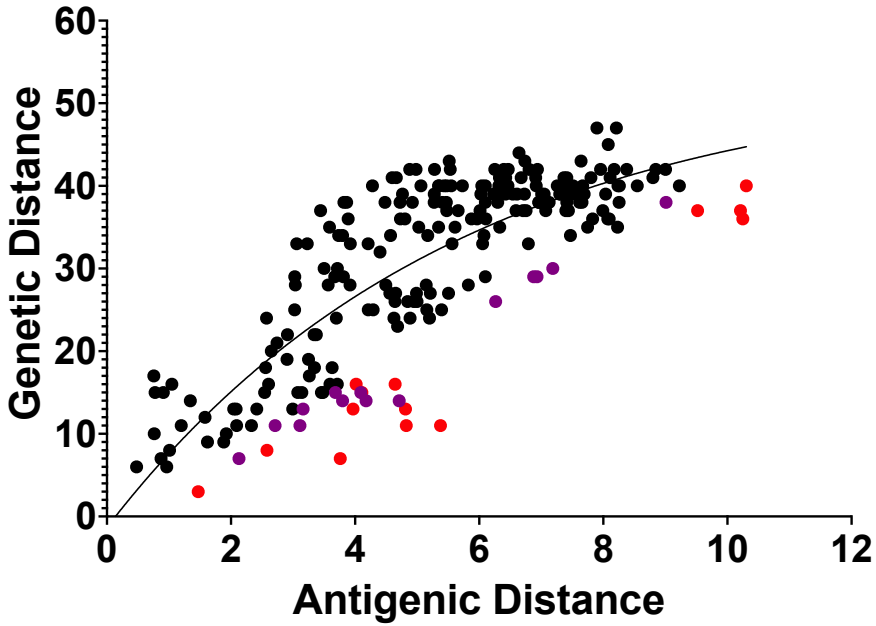

***Figure 2**: Virus pairwise plots of antigenic distance and genetic variation. Predicted residues taken from coloured plots.*

Results Continued

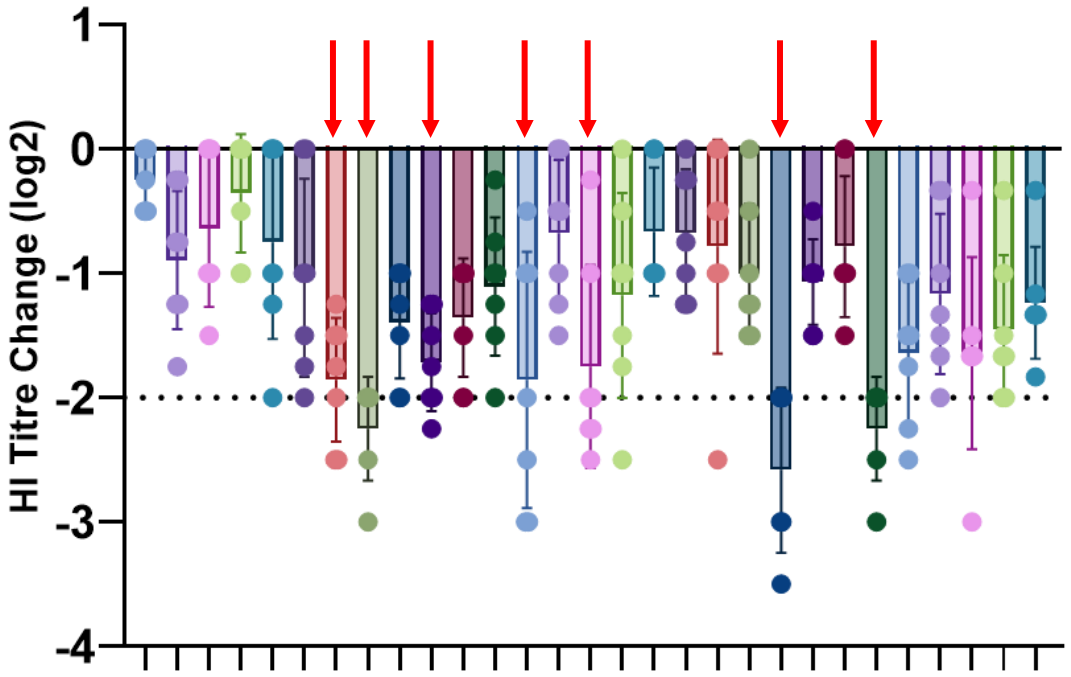

***Figure 3**: HI titres of putative epitopes. Arrows indicate antigenic epitopes (>2log2 HI titre change).*

Each predicted residue was introduced into a candidate HA and generated into an RG virus and assessed for antigenic influence by HI using homologous sera raised to the candidate HA.

All predicted residues impacted HI titre to some degree. Seven residues were significantly antigenic, three of which were novel.

Five of the mutants achieved HI titre change ≥3log2 which with poor antigenic matching, lead to vaccine failure in the field.

Conclusions

H5 clades carry significant antigenic heterogeneity with clade 2.3.4.4 presenting significant inter- and intra-clade diversities.

Our developed methods successfully identified multiple antigenic residues, some of which are novel, and will aid vaccine seed selection for poultry vaccines.

Putative epitopes showed antigenic variability with novel residue identification, which may confound vaccine efficacy.