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THE ANTIGENIC AND GENETIC EVOLUTION OF EQUINE INFLUENZA (H3N8)

INTRODUCTION

Equine influenza viruses are highly contagious and can lead to considerable economic loss to the racing industry. The prototype equine H3N8 virus was first isolated in 1963 and spread worldwide (1). Vaccination was introduced in the late 1960s and became mandatory for racehorses in 1981, but has failed to completely control disease. Here the technique of Antigenic Cartography (2) has been applied to equine influenza virus, to develop pictorial maps to facilitate analysis of the complex genetic and antigenic relationships between strains.

Aims:

- Quantify the antigenic and genetic evolution of equine influenza virus
- Determine the amino acids of antigenic significance in the evolution of equine influenza virus
- Improve vaccine strain selection

GENETIC EVOLUTION— four clusters representing sublineages

A genetic map based on haemagglutinin HA1 sequences has been generated for 151 strains (figure 1) and this has been compared to several phylogenetic trees of the same sequences as phylogenetic analysis has been traditionally used to evaluate strain evolutionary inter-relationships. The HA1 sequences have been aligned and compared to both a maximum likelihood tree (figure 2) showing the division into four sublineages, and the genetic map to elucidate the amino acid substitutions of significance in the evolution of the equine influenza virus into sublineages.

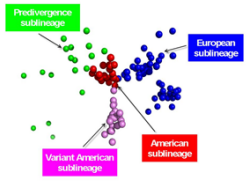


Figure 1

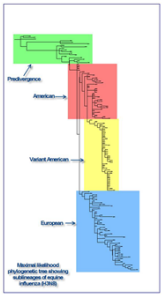


Figure 2

The results of the genetic analysis are shown in the flow chart (figure 3). Future work will aim to characterise the putative antigenic sites of equine influenza which from this sequential analysis appear to be similar but not identical to published antigenic sites of human H3 (3) .

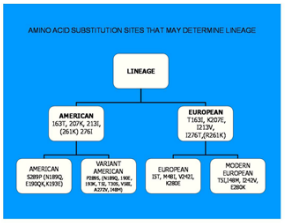
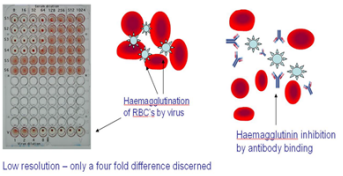


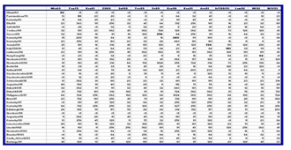
Figure 3

ANTIGENIC EVOLUTION— similarity to genetic map sublineages

Antigenic characterisation of influenza viruses is traditionally carried out using the haemagglutinin inhibition assay using a panel of polyclonal ferret sera. This test is of low resolution and results in complex tables from which inter-relationships between viruses can be difficult to interpret.



The haemagglutinin inhibition assay



Part of table of HI assay results

An extensive haemagglutination-inhibition assay data table, generated from 188 virus strains and 37 sera, has been distilled into an antigenic 'map', (figure 4) allowing visual representation of the inter-relationships between different strains. HI titres to the polyclonal ferret sera (shown in cubes below) position the virus strains (spheres) on the map using the antigenic cartography algorithm. The antigenic map below shows the development of sublineages of H3N8 over time and the division between the currently circulating American (red) and European (blue) lineages. The more recent variant American (VA) isolates are shown in pink and two isolates from 1988, representing the predivergence sublineage, in green.
The equine antigenic map is least distorted in 3D unlike the map of the human H3N2 strain which can be constructed in 2D.
The grid onto which the map is superimposed represents a two-fold difference in HI titre.

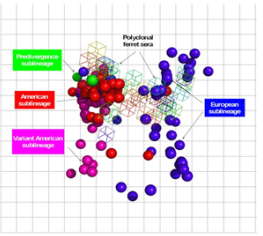


Figure 4

In 1988-1989 the European sublineage broke away from the predivergence sublineage viruses, which then continued to evolve as the American sublineage strains. The time series images from 1988 and 1989 (figures 5 and 6) demonstrate this dramatic jump.

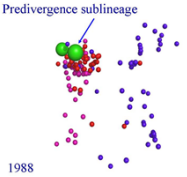


Figure 5

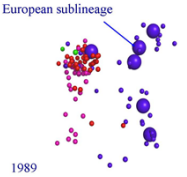


Figure 6

IMPROVING VACCINE STRAIN SELECTION

A serious outbreak in the UK and Europe in 2003 affected horses vaccinated as recently as 3 months earlier. This was the first time a representative of the variant American sublineage, as it is now known, had been isolated in the UK. The antibody level in acute sera in recently vaccinated horses was sufficiently high that it would have been expected to confer immunity to previously isolated American lineage viruses. However, it was not protective. The antigenic difference between the outbreak strain in Newmarket in 2003 and the current vaccine strain (A/eq/Newmarket/1/93) used by the majority of manufacturers for protection against the American lineage is demonstrated (figure 7).
The substitutions that may be significant between the Newmarket/1/93 vaccine strain and Newmarket/5/03 outbreak strain are shown on the HA structure (figure 8)(4).

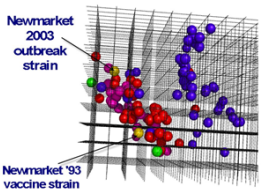


Figure 7

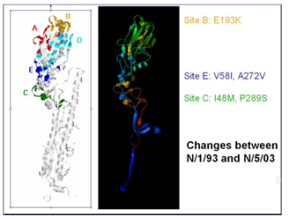


Figure 8

The current recommendations from the OIE (World Organization for Animal Health) suggest an update of American lineage vaccine strain to A/eq/South Africa/4/03. Isolates from North America within the VA sublineage seem to be evolving antigenically distinctly from the European VA recent isolates and this may have implications for vaccine strain selection in the future. Figure 9 shows the variant American sublineage isolates (circled) and the OIE recommended vaccine strain update virus.

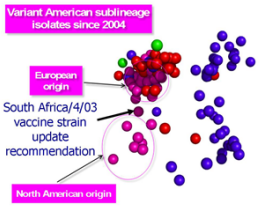


Figure 9

CONCLUSIONS

Antigenic cartography provides a powerful new tool to better understand the evolution of equine influenza so improving the value of surveillance data and will result in more accurate vaccine strain selection criteria in the future.

1. Gerber,H. (1970). "Equine influenza: Clinical features, sequealae and epidemiology of equine influenza." Proc.n dint. Conf. Equine infectious Diseases, Paris 1969, Karger:63-80.
2. Smith, D.J., Lapedes, A.S., de Jong, J.C., Bestebroer, T.M., Rimmelzwaan,G.F., Osterhaus, A.D.M.E. and Fouchier, R.A.M. (2004) "Mapping the antigenic and genetic evolution of influenza virus." Science 305: (5682) 371-6.
3. Daniels,R.S., Skehel,J.J and Wiley,D.C. (1985) "Amino acid sequences of haemagglutinins of influenza viruses of the H3 subtype isolated from horses." J.Gen. Virol 66:457-464
4. http://www.astex-therapeutics.com/AstexViewer/index.php

PyMOL http://www.pymol.org

cidc Cambridge Infectious Diseases Consortium