

## Brief report

# Potential of a sequence-based antigenic distance measure to indicate equine influenza vaccine strain efficacy<sup>☆</sup>

Janet M. Daly<sup>a,\*</sup>, Debra Elton<sup>b</sup><sup>a</sup> School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington LE12 5RD, UK<sup>b</sup> Centre for Preventive Medicine, Animal Health Trust, Newmarket, Suffolk CB8 7UU, UK

## ARTICLE INFO

## Article history:

Received 1 April 2013

Received in revised form 12 May 2013

Accepted 20 June 2013

Available online 2 July 2013

## Keywords:

Equine

Influenza

Vaccine

Efficacy

## ABSTRACT

The calculation of  $p_{\text{epitope}}$  values, a sequence-based measure of antigenic distance between strains, was developed for human influenza. The potential to apply the  $p_{\text{epitope}}$  value to equine influenza vaccine strain selection was assessed. There was a negative correlation between  $p_{\text{epitope}}$  value and vaccine efficacy for pairs of vaccine and challenge strains used in cross-protection studies in ponies that just reached statistical significance ( $p=0.046$ ) only if one pair of viruses was excluded from the analysis. Thus the  $p_{\text{epitope}}$  value has potential to provide additional data to consider in the decision-making process for updating equine influenza vaccine strains. However, further work is required to define the epitopes of the equine H3N8 haemagglutinin protein recognised by equine antibodies, which could lead to refinement of the  $p_{\text{epitope}}$  value calculation. Furthermore, other factors such as vaccine potency and virulence of circulating strains may also influence vaccine efficacy.

© 2013 The Authors. Published by Elsevier Ltd. All rights reserved.

## 1. Introduction

Equine influenza is a highly contagious upper respiratory tract infection caused by influenza virus type A. Vaccines against equine influenza have been in use since the 1960s; they rely on stimulation of antibodies to the surface glycoproteins of the virus, in particular the haemagglutinin (HA); binding to the HA blocks attachment of the virus to receptors on host cells. However, continual evolution of the viral proteins resulting in changes in the antibody-binding domains (antigenic sites) diminishes the ability of viruses raised to an earlier strain to neutralise a strain that has undergone ‘antigenic drift’ [1]. After major outbreaks caused by H3N8 subtype equine influenza in 1979–1980, mandatory vaccination of competition horses in the UK, Ireland and France was introduced, and A/equine/Fontainebleau/79 (H3N8) or A/equine/Kentucky/81 (H3N8) strains were included in vaccines with the H3N8 prototype strain A/equine/Miami/63. Having initially evolved in a single lineage [2], H3N8 viruses diverged into two lineages (‘Eurasian’ and ‘American’) in the late 1980s [3]. More recently, sub-lineages of the American lineage have emerged [4] and distinct clades within the sub-lineages [5].

A formal vaccine strain selection system for equine influenza vaccines modelled on the process for human influenza vaccine strain selection has been in operation for several years. The current recommendation of the OIE Expert Surveillance Panel on Equine Influenza Vaccine Composition is that equine influenza vaccines for international use should include two H3N8 subtype strains – a representative of clade 1 of the Florida sub-lineage (A/equine/South Africa/4/03, A/equine/Ohio/03 or other similar strain) and an A/equine/Richmond/1/07-like virus to represent clade 2 [6].

The decision to recommend a vaccine strain update is based primarily on a combination of antigenic data from haemagglutination inhibition (HI) assays using ferret antisera and sequencing of the HA1 portion of the HA, which encodes the major antibody-binding (antigenic) sites. Additionally, if available, field or experimental evidence of vaccine breakdown is taken into consideration. Sequence data offers several potential advantages over antigenic analysis using ferret antisera, not least of which is that it can be rapidly obtained. Previously, it was suggested for human influenza that a minimum of 4 or 5 amino acid changes in at least two antigenic sites would indicate a new antigenic variant [7]. However, studies have since shown that as few as one or two amino acid substitutions may compromise the effectiveness of vaccination sufficiently for epidemics to occur [8]. Mathematical modelling has suggested that the same may be true for equine influenza viruses [9]. However, there is, to date, insufficient information to reliably predict individual or combinations of amino acid changes that would lead to vaccine breakdown. Nonetheless, a sequence-based antigenic distance measure (the  $p_{\text{epitope}}$  value) for human influenza A viruses

<sup>☆</sup> This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial-No Derivative Works License, which permits non-commercial use, distribution, and reproduction in any medium, provided the original author and source are credited.

\* Corresponding author. Tel.: +44 0115 95 16475.

E-mail address: [janet.daly@nottingham.ac.uk](mailto:janet.daly@nottingham.ac.uk) (J.M. Daly).

**Table 1**  
Vaccine efficacy and  $p_{\text{epitope}}$  values for pairs of vaccine strains and challenge strains used in experimental cross-protection studies.

| Vaccine strain <sup>a</sup> | Challenge strain | No. ponies | SRH antibody (mm <sup>2</sup> ) |                   | Vaccine efficacy | $p_{\text{epitope}}$ (dominant site) | Total amino acid changes |
|-----------------------------|------------------|------------|---------------------------------|-------------------|------------------|--------------------------------------|--------------------------|
|                             |                  |            | Hom. <sup>b</sup>               | Het. <sup>c</sup> |                  |                                      |                          |
| Miami/63                    |                  | 10         | 126                             | 78                | 10%              | 0.333 (B)                            | 33                       |
| Fontainebleau/79            | Sussex/89        | 10         | 52                              | 18                | 10%              | 0.191 (B)                            | 17                       |
| Kentucky/81                 |                  | 10         | 74                              | 40                | 20%              | 0.148 (C)                            | 13                       |
| Suffolk/89                  |                  | 9          | 75                              | 46                | 44%              | 0.074 (C)                            | 3                        |
| Arundel/91                  | Newmarket/2/93   | 12         | 159                             | 120               | 33%              | 0.111 (C)                            | 8                        |
| Newmarket/1/93              |                  | 10         | 167                             | 119               | 40%              | 0.191 (B)                            | 12                       |
| Newmarket/2/93              | Newmarket/1/93   | 13         | 132                             | 113               | 15%              | 0.191 (B)                            | 12                       |

<sup>a</sup> GenBank accession numbers: A/equine/Miami/63 (M29257); A/equine/Fontainebleau/79 (CY032405); A/equine/Kentucky/1/81 (CY028820); A/equine/Suffolk/89 (X68437); A/equine/Sussex/89 (X85090); A/equine/Arundel/91 (X85085); A/equine/Newmarket/1/93 (X85088); A/equine/Newmarket/2/93 (X85089).

<sup>b</sup> Homologous antigen.  
<sup>c</sup> Heterologous antigen.

has been developed for use in adjunct with other data. Reasonable correlation between  $p_{\text{epitope}}$  values and vaccine effectiveness was demonstrated for human influenza A viruses of the H3N2 subtype [10–12] and has also been applied to the H1N1 subtype of human influenza [13].

## 2. Materials and methods

In order to assess the potential for the sequence-based antigenic measure to be applied in vaccine strain selection for equine influenza vaccines, the epitope values of various pairs of equine influenza virus isolates was calculated using the Excel file made available by Gupta et al. [11] (<http://www.mwdeem.rice.edu/pepitope>). The  $p_{\text{epitope}}$  value is calculated as the fraction of substituted amino acids in the dominant HA epitope, thus  $p$ -values are obtained for each of the five antigenic sites of the HA molecule and the highest value taken as the  $p_{\text{epitope}}$  value. The antigenic sites are those identified for the human influenza A H3 HA molecule [11]. These were used without modification as it has previously been suggested that the antigenic sites of the equine influenza H3 HA correspond with those of the human influenza H3 HA [14].

The strains analysed were those for which measures of vaccine efficacy were available from experimental cross-protection studies in ponies, most of which were vaccine strains or were assessed as candidate vaccine strains [9,15–17]. Vaccine efficacy was determined as the percentage of individuals fully protected from virus shedding (measured by titration of nasopharyngeal swab extracts in embryonated hens' eggs) after challenge. This is a more stringent definition of vaccine efficacy than that used by Gupta et al. [11] who used published data from various sources on the effectiveness of human influenza vaccines at preventing influenza-like illness.

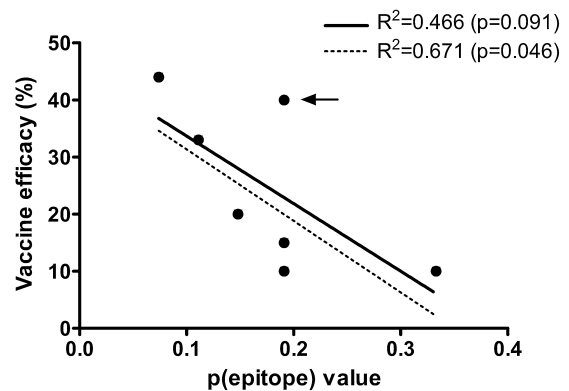
## 3. Results and discussion

Overall, the correlation between  $p_{\text{epitope}}$  value and vaccine efficacy was not significant (linear regression analysis with least squares estimation,  $R^2 = 0.2317$ ,  $p = 0.274$ ; Fig. 1). However, there was a notable discrepancy in vaccine efficacy for the only virus pair for which the reciprocal challenge was carried out (Newmarket/1/93 and Newmarket/2/93); the  $p_{\text{epitope}}$  value for this pair was 0.191 (Table 1). Whereas challenge of Newmarket/2/93-vaccinated horses with the Newmarket/1/93 strain gave a vaccine efficacy of 10%, only 4 of 10 ponies vaccinated with the Newmarket/1/93 strain and challenged with Newmarket/2/93 shed virus, giving a vaccine efficacy of 40%. Fontainebleau/79 and Sussex/89 have the same  $p_{\text{epitope}}$  value as Newmarket/1/93 and Newmarket/2/93 and the same immunodominant epitope (site B). Vaccine efficacy for this

pair was 10%, similar to that for the Newmarket pair when Newmarket/1/93 was the challenge strain, suggesting that the vaccine efficacy for the Newmarket/2/93 challenge is the outlier.

One potential explanation for an aberrantly high vaccine efficacy is a markedly more immunogenic vaccine preparation. The single radial immunodiffusion (SRID) assay is recommended for standardisation of the haemagglutinin content of equine influenza vaccines [18], but homologous standards were not available for most of the vaccine strains at the time the studies were carried out. Pre-challenge antibody levels were measured using the single radial haemolysis (SRH) assay [18]. Although the mean SRH values obtained with the heterologous strain were similar for the Newmarket/1/93 and Newmarket/2/93 vaccine groups, the mean homologous value was much higher for the Newmarket/1/93 vaccine group (167 mm<sup>2</sup> vs 132 mm<sup>2</sup>). Furthermore, 7 of the 10 ponies in the Newmarket/1/93 vaccine group (70%) had homologous pre-challenge antibody levels of greater than 150 mm<sup>2</sup> (the threshold value previously shown to be associated with protection against a heterologous challenge); [19] compared to only 3 of 13 ponies (23%) in the Newmarket/2/93 vaccine group.

A second potential explanation for the apparently higher efficacy of the Newmarket/1/93 vaccine is that the challenge was less rigorous. The Newmarket/2/93 strain was also used to challenge Arundel/91-vaccinated ponies; all control ponies shed virus in both Newmarket/2/93 challenge studies. However, a higher challenge dose was used for the Newmarket/2/93 challenge compared to the Newmarket/1/93 challenge (a total of  $10^{9.2}$  50% egg infectious doses vs  $10^{7.7}$  EID<sub>50</sub>) because in preliminary studies, the Newmarket/2/93 strain failed to elicit clinical signs in some seronegative ponies at



**Fig. 1.** Correlation between  $p_{\text{epitope}}$  and vaccine efficacy. The correlation between  $p_{\text{epitope}}$  and vaccine efficacy is shown with (solid line) and without (dotted line) the data point for Newmarket/1/93 vaccine and Newmarket/2/93 challenge (indicated by an arrow).

the lower challenge dose [15]. The Newmarket/2/93 strain was also shown to be a poorer inducer of pro-inflammatory cytokines than the Sussex/89 strain [20]. Thus the lower virulence of the Newmarket/2/93 strain may have been a contributory factor to the unexpectedly high vaccine efficacy together with the higher antibody levels seen in the Newmarket/1/93-vaccinated ponies. When the Newmarket/1/93 vaccine and Newmarket/2/93 challenge was excluded from the data set, the correlation between  $p_{\text{epitope}}$  and vaccine efficacy was significant ( $R^2 = 0.6714$ ,  $p = 0.046$ ) and a  $p_{\text{epitope}}$  value  $>0.2$  would appear to be strong indicator that vaccine efficacy is compromised (*i.e.*  $\leq 10\%$ ).

In conclusion, by excluding one pair of viruses, a marginally significant correlation was seen between the proportion of amino acid changes in the immunodominant epitope and vaccine efficacy for equine influenza A H3N8 strains. In contrast, there was no significant correlation between total number of amino acid changes between strains and vaccine efficacy ( $R^2 = 0.6390$ ,  $p = 0.07$ ). Although it may be necessary to take other factors, such as virulence of a circulating strain, into consideration, further refinement of the epitope analysis may be achieved by mapping the residues of the equine H3 HA targeted by neutralising antibodies in the horse and assigning greater weight in the calculation of the  $p_{\text{epitope}}$  value to particularly immunodominant residues.

### Acknowledgement

The data included in this work was available as a result of generous funding from the Horserace Betting Levy Board.

### References

- [1] Elton D, Bryant N. Facing the threat of equine influenza. *Equine Vet J* 2011;43:250–8.
- [2] Kawaoka Y, Bean WJ, Webster RG. Evolution of the hemagglutinin of equine H3 influenza viruses. *Virology* 1989;169:283–92.
- [3] Daly J, Lai A, Binns M, Chambers T, Barrandeguy M, Mumford J. Antigenic and genetic evolution of equine H3N8 influenza A viruses. *J Gen Virol* 1996;77:661–71.
- [4] Lai AC, Rogers KM, Glaser A, Tudor L, Chambers T. Alternate circulation of recent equine-2 influenza viruses (H3N8) from two distinct lineages in the United States. *Virus Res* 2004;100:159–64.
- [5] Bryant NA, Rash AS, Russell CA, Ross J, Cooke A, Bowman S, et al. Antigenic and genetic variations in European and North American equine influenza virus strains (H3N8) isolated from 2006 to 2007. *Vet Microbiol* 2009;138:41–52.
- [6] Anon. OIE expert surveillance panel on equine influenza vaccine composition. *OIE Bulletin*; 2012. p. 46–9.
- [7] Wilson IA, Cox NJ. Structural basis of immune recognition of influenza virus hemagglutinin. *Annu Rev Immunol* 1990;8:737–71.
- [8] Jin H, Zhou H, Liu H, Chan W, Adhikary L, Mahmood K, et al. Two residues in the hemagglutinin of A/Fujian/411/02-like influenza viruses are responsible for antigenic drift from A/Panama/2007/99. *Virology* 2005;336:113–9.
- [9] Park AW, Daly JM, Lewis NS, Smith DJ, Wood JL, Grenfell BT. Quantifying the impact of immune escape on transmission dynamics of influenza. *Science* 2009;326:726–8.
- [10] Deem MW, Lee HY. Sequence space localization in the immune system response to vaccination and disease. *Phys Rev Lett* 2003;91:068101.
- [11] Gupta V, Earl DJ, Deem MW. Quantifying influenza vaccine efficacy and antigenic distance. *Vaccine* 2006;24:3881–8.
- [12] Lee MS, Chen JS. Predicting antigenic variants of influenza A/H3N2 viruses. *Emerg Infect Dis* 2004;10:1385–90.
- [13] Pan K, Subieta KC, Deem MW. A novel sequence-based antigenic distance measure for H1N1, with application to vaccine effectiveness and the selection of vaccine strains. *PEDS* 2011;24:291–9.
- [14] Daniels RS, Skehel JJ, Wiley DC. Amino acid sequences of haemagglutinins of influenza viruses of the H3 subtype isolated from horses. *J Gen Virol* 1985;66:457–64.
- [15] Daly JM, Yates PJ, Newton JR, Park A, Henley W, Wood JL, et al. Evidence supporting the inclusion of strains from each of the two co-circulating lineages of H3N8 equine influenza virus in vaccines. *Vaccine* 2004;22:4101–9.
- [16] Daly JM, Yates RJ, Browse G, Swann Z, Newton JR, Jessett D, et al. Comparison of hamster and pony challenge models for evaluation of effect of antigenic drift on cross protection afforded by equine influenza vaccines. *Equine Vet J* 2003;35:458–62.
- [17] Yates P, Mumford JA. Equine influenza vaccine efficacy: the significance of antigenic variation. *Vet Microbiol* 2000;74:173–7.
- [18] OIE. Equine influenza. In: *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. 7th ed; 2012.
- [19] Mumford JA, Wood J. Establishing an acceptability threshold for equine influenza vaccines. *Dev Biol Stand* 1992;79:137–46.
- [20] Watrang E, Jessett DM, Yates P, Fuxler L, Hannant D. Experimental infection of ponies with equine influenza A2 (H3N8) virus strains of different pathogenicity elicits varying interferon and interleukin-6 responses. *Viral Immunol* 2003;16:57–67.