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Brief report

Potential of a sequence-based antigenic distance measure to indicate equine influenza vaccine strain efficacy[☆]

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ABSTRACT

The calculation of p_{epitope} values, a sequence-based measure of antigenic distance between strains, was developed for human influenza. The potential to apply the p_{epitope} value to equine influenza vaccine strain selection was assessed. There was a negative correlation between p_{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_{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_{epitope} value calculation. Furthermore, other factors such as vaccine potency and virulence of circulating strains may also influence vaccine efficacy.

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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_{epitope} value) for human influenza A viruses

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

Vaccine strain ^a	Challenge strain	No. ponies	SRH antibody (mm ²)		Vaccine efficacy	p_{epitope} (dominant site)	Total amino acid changes
			Hom. ^b	Het. ^c			
Miami/63	Sussex/89	10	126	78	10%	0.333 (B)	33
Fontainebleau/79		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

^a 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).

^b Homologous antigen.
^c Heterologous antigen.

has been developed for use in adjunct with other data. Reasonable correlation between p_{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_{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_{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_{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_{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_{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 mm² vs 132 mm²). Furthermore, 7 of the 10 ponies in the Newmarket/1/93 vaccine group (70%) had homologous pre-challenge antibody levels of greater than 150 mm² (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 $10^{7.7}$ EID₅₀) because in preliminary studies, the Newmarket/2/93 strain failed to elicit clinical signs in some seronegative ponies at

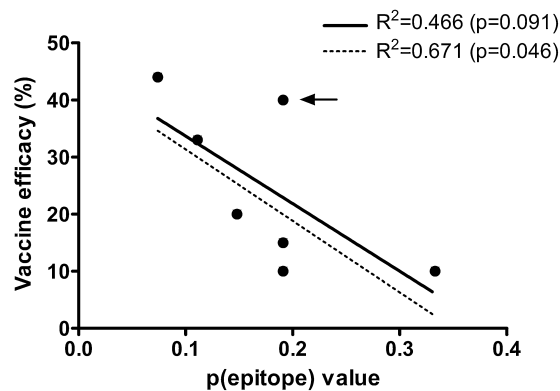


Fig. 1. Correlation between p_{epitope} and vaccine efficacy. The correlation between p_{epitope} and vaccine efficacy is shown with (solid line) and without (dotted line) the data point for Newmarket/1/93 vaccine and Newmarket/2/93 challenge (indicated by an arrow).

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_{epitope} and vaccine efficacy was significant ($R^2 = 0.6714$, $p = 0.046$) and a p_{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_{epitope} value to particularly immunodominant residues.

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