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Large scale sero-epidemiological investigation of Equine Influenza vaccination in Hong Kong

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Equine influenza (EI) is a major respiratory disease of the horse induced by the highly contagious equine influenza virus (EIV). Worldwide, diverse EI vaccines are available commercially. The EIV strain, adjuvant composition and vaccine technology differs greatly. The use of the same EI vaccine throughout the life of a horse is unlikely, due to change of ownership, veterinary practitioner, location and/or vaccine availability. The compatibility of different EI vaccines has rarely been studied but is of great importance as immunisation failure could favour EIV dissemination. Study aims: To determine the impact of mixed EI vaccination on protective immunity to EIV in horses and to measure herd immunity in Hong Kong (HK). Methods: HK has approximately 1,250 race horses with around 450 new horses arriving each year. Imported racehorses must have been vaccinated with an approved EI vaccine, irrespective of their point of origin. All horses are given a new primary course on arrival with a unique ISCOM matrix adjuvanted EI vaccine and are vaccinated twice a year thereafter. EI-specific antibody titres were measured by single radial haemolysis (SRH) assay (A/eq/South Africa/4/03 EIV strain) in 1050+ archived sera collected between 2010 and 2014 from 129+ horses. Objectives: 1) to determine the SRH antibody titre in serums from HK resident horses (i.e. in HK for at least 1.5 year), 2) to evaluate the antibody titre on arrival at HK in relation to their country of origin, 3) to measure the effect of post-arrival mandatory EI primary vaccination and 4) to compare this response with antibody levels measured in HK resident horses. Results: Preliminary results indicates that the average yearly SRH antibody titre for HK resident horses was 113±34mm^2 (n=467), with only 15% of horses below the clinical protection threshold (i.e. 85mm^2) and less than 3% of horses below 50mm^2. A significant increase was detectable (p-value<0.00004) after each immunisation boost (maximum average titre of 128±42mm^2 in October, n=55). The average SRH antibody titre in the week following importation to HK (and prior the mandatory EI vaccination in HK) was 180±40mm^2 (n=79), with results in horses originating in Australia significantly higher (207±36mm^2, n=10, p-value = 0.0281) than horses from New Zealand (171±34mm^2, n=42), Great Britain (168±38mm^2, n=17) or Ireland (172±31mm^2, n=3). SRH antibody titres at the time of importation were maintained for around 75 days (Figure 1) due to post-importation immunisation before decreasing slowly to reach the levels of HK resident horses (around 200-225 days post importation). Conclusion: There are few large scale sero-epidemiological investigations monitoring horses after EI vaccination. Preliminary analysis indicates that a large proportion (85%) of the HK horse population has protective SRH antibody titre, which should provide efficient herd immunity and protection against EI. The impact of mixed EI immunisation is currently being analysed.

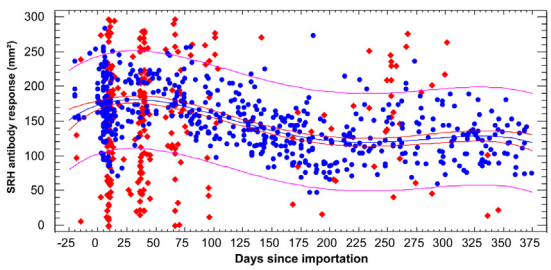

Figure 1: SRH antibody response after importation to HK (Day=0). Blue dots correspond to samples analysed (n=577), red diamonds correspond to vaccination points (n=264).

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Refinement of the Equine Influenza model: the benefits of individual nebulisation for experimental infection

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Equine Influenza (EI) is an important respiratory disease of the horses. Numerous EI vaccines are commercially available worldwide and an accurate evaluation of their efficacy is required through clinical trials conducted in the natural host challenged by experimental infection with equine influenza virus (EIV). Room nebulisation is one of the recognised/chosen methods to deliver an EIV infectious aerosol during the *in vivo* challenge phase of EI vaccine studies. This method has been frequently used in the last few decades; it is well tolerated, requires minimal animal manipulation and has a demonstrated efficacy. However, it presents inherent limitation, such as the difficulty to accurately determine the EIV dose inhaled by an individual animal. Furthermore, the suspected decreased pathogenicity of recent Florida Clade 2 (FC2) EIV isolates have increased the

heterogeneity of the clinical response and virus shedding measured after infection by room nebulisation. This situation raises concerns, as it could impact the statistical power of a study and may require increasing the number of animals per group in future studies. Objectives: This report aims to evaluate individual nebulisation as a refinement of the experimental EIV infection model. Methods: Meta-analysis of clinical examination records and virus shedding measured in naïve Welsh mountain ponies experimentally infected with the FC2 EIV strains A/eq/Cambremer/2012 and A/eq/Northamptonshire/1/13 by room or individual nebulisation. Results from 5 independent studies were analysed. Results: Experimental infection by room nebulisation using the EIV strain A/eq/Cambremer/2012 revealed a notable heterogeneity of the clinical and virological responses between studies. Overall, the homogeneity of these responses was clearly improved when the EIV A/eq/Cambremer/2012 infectious dose was delivered by individual nebulisation. This result was confirmed when 2 studies, using the EIV A/eq/Northamptonshire/1/13 strains delivered by individual nebulisation, were compared. Advantages/disadvantages of individual nebulisation when compared to room nebulisation: More controlled dose delivered and better homogeneity of the response measured in control animals. The individual nebuliser used in these studies (FlexiNeb) showed great improvements when compared to previous models described, being noiseless, self-contained, light, resistant, with a rapid delivery of the infectious dose (<2min), no requirement to sedate the animal and is easy to decontaminate. However, animals do require some acclimatisation prior to infection. Conclusion: This report analyses the clinical and virological responses induced by room or individual EIV nebulisation. It reviews the advantages and disadvantages of this nebulisation methods used for the experimental EIV infection model. Individual nebulisation proved to be efficient and effective for the experimental infection of ponies with EIV, which helps to maintain the number of animals per group to the minimum necessary and required in order to obtain meaningful results, which adheres to the 3R's (Replace, Reduce and Refine) principles.

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Molecular dynamics of influenza A virus adaptation in horses

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Influenza A viruses (IAVs) pose a constant threat to humans and animals and horses are no exception to this rule. In the last 60 years, horse populations have been invaded by at least three distinct IAVs of likely avian origin. The first equine influenza virus (EIV) to be isolated was an H7N7 IAV that circulated for approximately 20 years before becoming extinct. The second EIV to be reported was an H3N8 virus that originated in South America and is still circulating in horses despite the availability of vaccines. A third EIV was isolated in Jilin, China, during a large epizootic with high morbidity and mortality. This virus, which only circulated for a couple of years, was also of the H3N8 subtype, albeit phylogenetically different from the "classical" EIV discovered in 1963. Evolution plays a key role in post-transfer adaptation during the establishment of emergent viruses in new host species. However, the molecular mechanisms that underpin this process are not known. Here, we combined phylogenetics with *in vitro* assays and reverse genetics technology to examine the evolutionary dynamics of EIV adaptation from a functional perspective. We excluded antigenic drift from our analysis as the antigenic evolution of EIV has already been studied. Our results show that EIV post-transfer adaptation is a multigenic trait and that evolutionary distinct EIVs exhibited different strategies to overcome the host innate immune response. We also observed that the efficiency in genome replication changed over time. Finally, we examined the transcriptome of equine cells infected with adapted or non-adapted H3N8 IAVs to explore the intracellular pathways that might be important during IAV adaptation to the horse.

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Analysis of codon usage bias and evolution of equine influenza viruses

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Equine influenza viruses (EIVs) cause severe respiratory disease in horses across the world. EIV is classified as influenza type A virus under family *Orthomyxoviridae.* Like other influenza viruses, EIV also evolved into various lineages and sub-lineages which are circulating globally. The cross-species jumping of equine origin H3N8 has also been observed to infect dogs and pigs. As influenza viruses completely dependent on host cellular machinery for their replication, their adaptability in a particular host is significantly influenced by codon usage of virus. Therefore, understanding of codon usage of EIVs will help in elucidating the mechanism of evolution and host adoption. In present study, we analyzed genome-wide codon usage patterns in 92 EIVs including both H3N8 and H7N7 subtypes by computing several codon usage indices. The complete genome sequences of EIVs isolated during 1963-2013, were retrieved from the "Flu database, NCBI" and data set was generated by concatenating the open reading frames (ORFs) of each strain. The nucleotide composition analysis revealed that A/U-ended codons might be preferred over G/C-ended codons in EIVs. The relative synonymous codon usage (RSCU) values of each codon revealed that among 18 most abundantly used codons in EIVs, 14 were A/U-ended and 4 were G/C-ended codons in H3N8 genotypes, whereas patterns of synonymous codon usage in H7N7 genotypes was quite different. Similar finding also reported in H5N1, H1N1 and H3N2 viruses. We also noticed close homology in RSCU pattern of horse and dog compared to donkey, despite belonging to same *Equidae* (horse and donkey) family. The abundance of CpG dinucleotide in EIV genomes was low which contribute in codon usage bias towards escaping the host antiviral immune response, as low unmethylated CpG is available for stimulating host innate immune system. The ENc analysis revealed high codon usage bias in H7N7 than H3N8. Furthermore, we compared the codon usage pReferences of EIVs with respect to different host species using codon adaptation index (CAI). It is interesting to note that CAI of H3N8 isolated from horse and canine were identical. The similarity also found in other indices values of canine H3N8 with equine H3N8. This clearly suggests that the equine H3N8 crossed the species barrier and started infecting the canines. Besides, codon usage pReferences of haemaggutinin and neuraminidase were low compared to other genes, because of their less opportunity for interaction with host factors. The ENc-plot and neutrality plot analysis indicated that the mutational pressure is not the sole factor in shaping the codon bias, but other factors also involved in the process. Possible explanation of extinction of H7N7 might be