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Refinement of the equine influenza model in the natural host: A meta-analysis to determine the benefits of individual nebulisation for experimental infection and vaccine evaluation in the face of decreased strain pathogenicity

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ABSTRACT

Equine Influenza (EI) is an important respiratory disease of horses caused by H3N8 equine influenza viruses (EIV). Vaccination is a key strategy to prevent or control this disease. However, EIV undergoes continuous antigenic drift and whilst numerous EI vaccines are commercially available worldwide, an accurate evaluation of their efficacy is frequently required through clinical trials conducted in the natural host. Room nebulisation is one of the chosen methods to challenge horses during EI vaccine studies. A potential decreased pathogenicity observed with recent Florida Clade 2 (FC2) EIV isolates have increased the heterogeneity of the clinical response and virus shedding measured after infection by room nebulisation, which reduced the statistical power of studies. Our objectives were to compare clinical and virological parameters following experimental infection with several different EIV strains and to confirm that individual nebulisation is a model refinement that prevents an increase of the number of animals per group. This study is a retrospective comparison and meta-analysis of clinical and virological results collected from 9 independent EIV infection studies in the natural host. Naïve Welsh mountain ponies were experimentally infected by room or individual nebulisation with FC2 EIV strains, including A/equine/Richmond/1/07 (R/07), A/equine/East Renfrewshire/11 (ER/11), A/equine/Cambremer/1/2012 (C/12) and A/equine/Northamptonshire/1/13 (N/1/13). The retrospective meta-analysis confirmed a decreased pathogenicity of the EIV ER/11 and C/12 strains when compared with R/07. Experimental infection by individual nebulisation improved the clinical and virological parameters induced by recent FC2 strains, when compared with conventional room nebulisation. In conclusion, individual nebulisation offers a better control of the challenge dose administered and a greater homogeneity of the response measured in control animals. This in turn, helps maintain the number of animals per group to the minimum necessary required to obtain meaningful results in vaccine efficacy studies, which adheres to the 3Rs (Replacement, Reduction and Refinement) principles.

Abbreviations: AHT, Animal Health Trust; EI, equine influenza; EIV, equine influenza virus; ESP, equine surveillance panel; FC1 or FC2 respectively, Florida clade 1 or clade 2; 3Rs, replacement reduction and refinement

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1. Introduction

Equine influenza (EI) is a highly contagious respiratory disease of equids. Clinical signs of disease in naïve horses are characterised by an elevation of body temperature, nasal discharge, cough, and sometimes respiratory distress. Equine influenza is associated with high morbidity and, in some cases mortality of immunocompromised individuals (Landolt et al., 2014). This disease is caused by the inhalation of infectious equine influenza virus (EIV) aerosolised in high quantities during coughing episodes (Landolt et al., 2014). Equine influenza outbreaks have affected many countries and are important in terms of animal welfare. Their size could be significant in an unvaccinated population and they may have a profound economic impact on the horse racing/breeding industries (Paillot, 2014). A decade ago, Australia suffered a major EI epizooty, with around 75,000 horses affected and an estimated cost of A$1billion to the Australian economy (reviewed in (Smyth et al., 2011; Paillot and El-Hage, 2016)).

Increasing international movement of horses and occasional EIV outbreaks highlight the need for good quarantine and/or preventive measures such as vaccination (Paillot, 2014). Currently, several EI vaccines are commercially available worldwide (Paillot, 2014) but the continuous evolution of EIV through mutations (i.e. antigenic drift (Legrand et al., 2013; Woodward et al., 2014; Fougerolle et al., 2017; Rash et al., 2017)) and potential apparition of immune escape mutants requires a frequent evaluation of EI vaccines efficacy in the natural host. Efficacy studies are frequently conducted at the onset of vaccine immunity, when significant protection has to be demonstrated. Occasionally, duration of immunity is also investigated. Efficacy studies are used to evaluate, improve and/or register experimental and commercially available EI vaccines (reviewed in (Paillot et al., 2006; Paillot, 2014)). The most common method for experimentally infecting equids with EIV involves administration of the challenge dose by room nebulisation (generation of EIV infectious particles within a contained space/room and inhalation during 15 to 20 min). This method has proved to be very effective at inducting clinical signs of EI and virus shedding in naïve animals, superior to other methods such as intranasopharyngeal instillation, it is well tolerated and requires minimal animal manipulation (Mumford et al., 1990).

Equine influenza virus currently circulating worldwide belongs to two genetically and antigenically different H3N8 clades of the Florida sub-lineage (Fig. 1), the Clades 1 (FC1; e.g. strain circulating in the Americas) and 2 (FC2; e.g. strains circulating in Europe) (Legrand et al., 2013; Woodward et al., 2014; Fougerolle et al., 2017; Rash et al., 2017). In 2010, inclusion of FC2 strains in EI vaccines was recommended by the OIE expert surveillance panel (ESP) (OIE, 2010) and several FC2 EIV strains were subsequently evaluated and used as challenge strains in EI vaccine efficacy studies (A/equine/Richmond/1/07 (R/07), A/equine/East Renfrewshire/2/11 (ER/11) and A/equine/Cambremer/1/2012 (C/12)) (Paillot et al., 2013a, 2013b, 2015; Pouwels et al., 2014). However, our preliminary review of results indicated that ER/11 and C/12 tended to display less pathogenicity than the representative FC2 isolate R/07 when delivered by room nebulisation, often limited to nasal discharge, cough and low/moderate fever in some control animals. This change in pathogenicity was at risk to affect the markers of infection (i.e. clinical signs of disease and virus shedding). As a result, the statistical power of efficacy studies may be reduced, which would require increasing the number of animals per group as a corrective action. Individual nebulisation was identified as an alternative solution to room nebulisation, in order to optimise the response to infection with recent FC2 EIV strains through delivery of a more accurate infectious dose with subsequent improved control and homogeneity of the clinical and virological infection markers.

This report corresponds to a meta-analysis and retrospective comparison of nine independent studies (control groups only) using four different FC2 EIV isolates as challenge strains. The primary objective was to confirm the decreased pathogenicity of recent FC2 EIV strains. The secondary objective was to demonstrate that individual nebulisation represents a refinement of the experimental EIV infection equine model, in order to compensate for the observed decreased pathogenicity and increased heterogeneity of the response observed.

2. Materials and methods

2.1. Studies and animals

2.1.1. Study design

This report is a retrospective meta-analysis of results from 9 independent and single-center EI studies (Table 1). Study #7 was specifically designed to evaluate feasibility of individual nebulisation. All other studies were carried out for non-related reasons over a period of 5 years. Studies #1 to #3 were previously published (Paillot et al., 2013a, 2013b, 2015). The experimental unit was defined as one pony.

2.1.2. Animal welfare

All animal studies were carried out under the UK Animals Scientific Procedures Act 1986, with the approval of the Animal Health Trust (AHT) Ethics Review Committee and Study Sponsors ethical review committee, where applicable. All procedures were conducted at the AHT.

2.1.3. Inclusion criteria

The animals used in these studies were Welsh mountain ponies. Their ages vary depending on the type of study but ranged between 5 and 20 months. All animals were seronegative for EIV at the study start and time of experimental infection (control group only), with no known history of EI vaccination or previous contact with EIV.

2.1.4. Setting, location and sample size

All ponies were housed on AHT premises during the studies, kept as one group, either at pasture on grass or in barns with nearby sampling facilities. Drinking water was available *ad libitum*. Animals were moved into a category II containment facility two days prior to day of challenge (acclimatisation) and for a minimum of 2 weeks post-infection. Sample size was dependent of the studies'. When conducted for evaluation of EI vaccine efficacy, sample size were based on power calculations and to meet the European Pharmacopoeia criteria for EI vaccine (inactivated), with no fewer than 6 and 4 horses for the treated and control groups, respectively (Anonymous, 2017). For studies #7 and #3 (Paillot et al., 2013b), sample size was limited to the minimal biological repeats (n = 3) and control of successful infection (n = 2), respectively.

2.1.5. Randomisation and masking

To reduce any study bias, all studies conducted were masked and randomised, except the pilot study #7 that was designed to test the use of an individual nebuliser. Several of the studies were randomised to comply with specific study objectives. The report of animal studies follows the ARRIVE and CONSORT guidelines (supplementary check lists) (Kilkenny et al., 2010; Moher et al., 2010; Schulz et al., 2010).

2.2. EIV strains

Viruses were all grown in 10-day-old embryonated hen's eggs, purified and titrated, as previously described (Paillot et al., 2013a). The EIV FC2 strains A/equine/Richmond/1/07 (R/07), A/equine/East Renfrewshire/2/11 (ER/11), A/equine/Cambremer/1/2012 (C/12) and A/equine/Northamptonshire/13 (N/1/13) were used as challenge strains (Fig. 1; Supplementary Table 1). Equine influenza virus titres were expressed as 50% egg infectious dose (EID_{50}) per ml (Reed and Muench, 1938).