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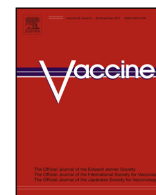

Humoral response and antiviral cytokine expression following vaccination of thoroughbred weanlings—A blinded comparison of commercially available vaccines

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ABSTRACT

Previous studies in experimental ponies using interferon gamma (IFN-γ) as a marker for cell mediated immune (CMI) response demonstrated an increase in IFN-γ gene expression following vaccination with an ISCOM subunit, a canarypox recombinant and more recently, an inactivated whole virus vaccine. The objective of this study was to carry out an independent comparison of both humoral antibody and CMI responses elicited following vaccination with all these vaccine presentation systems. Antibody response of 44 Thoroughbred weanlings was monitored for three weeks following the second dose of primary vaccination (V2) by single radial haemolysis (SRH). The pattern of antibody response was similar for all vaccines. The antibody response of horses vaccinated with the inactivated whole virus vaccine (Duvaxyn IE-T Plus) was superior to that of the horses vaccinated with the ISCOM-matrix subunit (Equilis Prequenza Te) and canarypox recombinant (ProteqFlu-Te) vaccine. In this study 39% of weanlings failed to seroconvert following their first dose of primary vaccination (V1). Poor response to vaccination (H3N8) was observed among weanlings vaccinated with Equilis Prequenza Te and ProteqFlu-Te but not among those vaccinated with Duvaxyn IE-T Plus. PAXgene bloods were collected on days 0, 2, 7 and 14 following V1. Gene expression levels of IFN-γ, IL-1β (proinflammatory cytokine) and IL-4 (B cell stimulating cytokine) were measured using RT-PCR. Mean gene expression levels of IL-1β and IL-4 peaked on day 14 post vaccination. The increase in IL-4 gene expression by horses vaccinated with Equilis Prequenza Te was significantly greater to those vaccinated with the other two products. Vaccination with all three vaccines resulted in a significant increase in IFN-γ gene expression which peaked at 7 days post V1. Overall, there was no significant difference in IFN-γ gene expression by the horses vaccinated with the whole inactivated, the subunit and the canarypox recombinant vaccines included in this study.

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1. Introduction

Equine influenza (EI) is endemic in Europe and North America and outbreaks of major economic significance have been reported on all continents. Despite the many different combinations of haemagglutinin (HA) and neuraminidase subtypes that occur in birds, only subtypes H7N7 and H3N8 have ever established in horses. H7N7 viruses caused outbreaks of influenza in horses for over two decades but are now considered extinct [1]. Since 1979 all outbreaks of EI for which there are virus isolates, have been attributed to H3N8 and the OIE (Office International des Epizooties) stipulates that there is no requirement for inclusion of a H7N7 virus in vaccines [2]. However, the majority of commercially available vaccines contain both virus subtypes. Vaccination against EI is an effective method of disease control in many countries worldwide. In countries where equine influenza virus (EIV) is endemic, vaccination minimises the incidence of disease and dissemination of virus at equestrian events. Since the introduction of mandatory EI vaccination of race horses in Ireland and the UK in the early 1980s, no race meeting or major equestrian event has been cancelled in either country as a result of the disease [3]. In countries where the virus is not endemic, vaccination in conjunction with quarantine are the barriers which prevent an incursion of the virus. Notwithstanding, in the past twenty five years major outbreaks have occurred in South Africa (1986 and 2003), India (1987), Hong Kong (1992) and more recently Australia (2007) following the international movement of horses vaccinated with suboptimal products that appear to have induced clinical but not virological protection [4–8].

Vaccine evaluations are carried out by vaccine companies or at the request of vaccine companies for regulatory or marketing purposes. These studies are usually performed under optimal conditions for vaccination and predominately in experimental ponies

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rather than target animals in the field. In order to best evaluate humoral response following vaccination, serum antibodies generated against the virus HA are commonly measured using the single radial haemolysis (SRH) test. These antibodies neutralise virus infectivity and antibody levels correlating with protection have been established in both experimental challenge studies and in the field [9–12]. A recent independent comparative vaccine study carried out in seronegative Thoroughbred weanlings demonstrated a significant difference in SRH antibody levels following vaccination with commercially available products [13]. Such independent evaluations are carried out infrequently and are essential for equivalent comparison between products.

While it is widely accepted that humoral antibody levels are fundamental for protection, there is evidence that immune mechanisms other than serum antibody may play a role in the prevention of disease. Protection against experimental challenge with EI was demonstrated six months after a single intranasal administration of a cold-adapted, modified live EI vaccine despite the absence of high antibody levels [14]. Other studies have shown that ponies with low antibody levels exhibited partial clinical protection when rechallenged up to 18 months following first infection, suggesting that immune mechanisms involving rapid stimulation of both B and T cells responses could assist in protection [15,16]. Studies carried out using interferon gamma (IFN-γ) protein synthesis as a marker for a cell mediated immune (CMI) response have demonstrated an increase in IFN-γ gene expression following vaccination with a canarypox recombinant [17,18], an Immuno Stimulating Complex (ISCOM) subunit [19], an ISCOM-matrix subunit [20] and an inactivated whole virus vaccine [21]. In this study real time RT-PCR was used to quantify cytokine gene expression. This method has been validated in the profiling of human and equine cytokines [22,23] but post transcriptional and post translational modifications may result in imperfect correlation between mRNA and protein synthesis. However a strong correlation between mRNA and secreted protein levels has been demonstrated for human IFN-γ following vaccination [24]. The objective of this study was to extend our previous comparative vaccine study in Thoroughbred horses and evaluate both the humoral antibody response and antiviral cytokine expression following vaccination with a whole virus (Duvaxyn IE-T Plus), a subunit ISCOM-matrix (Equilis Prequenza Te) and a canarypox recombinant vaccine (ProteqFlu-Te).

2. Material and methods

2.1. Horses

This study was carried out in a population of 44 unvaccinated Thoroughbred weanlings on a private stud farm. The weanlings ranged in age from 183 to 342 days (mean 259 ± 6.8 SE days) at the time of first vaccination (V1).

2.2. Vaccines

The whole virus vaccine Duvaxyn IE-T Plus (Elanco Animal Health), ISCOM-matrix subunit vaccine Equilis Prequenza Te (MSD Animal Health) and canarypox recombinant vaccine ProteqFlu-Te (Merial) included in this study were purchased commercially. The composition of these vaccines has previously been described [13].

2.3. Vaccination

The weanlings were randomly allocated one of the three vaccines and received two doses (V1 and V2) 29 days apart by deep intramuscular injection. Fourteen weanlings received Duvaxyn IE-T Plus, 15 received Equilis Prequenza Te and 15 received ProteqFlu-Te.

2.4. Collection of samples

Whole blood and PAXgene blood samples (PreAnalytix, Switzerland) were collected at V1, two, seven and 14 days post V1. Additional whole blood samples were collected at V2 and 21 days post V2.

2.5. Serology

Antibodies against A/eq/Newmarket/2/93 (H3N8), A/eq/Meath/07 (H3N8), A/eq/South Africa/4/03 (H3N8) and A/eq/Prague/56 (H7N7) were measured using the SRH test as previously described [13]. Seroconversion was defined as an increase in SRH antibody level of 25 mm^2 or 50% whichever was smaller between the paired serum samples [11]. A poor responder was defined as a horse that did not mount a mean H3N8 SRH antibody response of >25 mm^2 post vaccination [13].The laboratory investigator was blinded to vaccine allocation to individual horses.

2.6. Relative quantification of cytokine gene expression using real-time RT-PCR

PAXgene blood samples were processed as per the manufacturer's instructions and extracted RNA was quantified by measuring absorbance at 260 nm. Reverse transcription and quantitative real-time PCR reactions were carried out using the AgPath-ID One Step RT-PCR Kit (Ambion/Applied Biosystems, Foster City, CA, USA) on an ABI Taqman 7500 platform. Primer probe sets for the detection of IL-1β [25], IL-4, IFN-γ [18] and an endogenous control, β-GUS [25] were used as previously described. β-GUS is consistently expressed in experimental samples, does not have processed pseudogenes and is commonly used in equine cytokine expression studies [18,25–27].

Reverse transcription reactions were carried out using half a microgram of total cellular RNA. For IL-1β, each 25 μl reaction contained 5 μl RNA, 2× RT-PCR buffer, 80 ng tRNA (Sigma–Aldrich), 0.6 μM of each primer, 0.3 μM probe and 25× RT-PCR enzyme. For IL-4, IFN-γ, and β-GUS primer concentrations were 0.2 μM, 0.2 μM and 0.4 μM respectively.

Each sample was tested in triplicate for each of the cytokine targets and the endogenous control. One step RT- PCR was carried out at 45 °C for 10 min followed by 95 °C for 10 min, 45 cycles of 95 °C for 15 s and 60 °C for 60 s. Data were analysed as a relative quantification study with day 0 samples as the calibrator for each horse. Relative quantification was then used to compare gene expression levels post vaccination using the $2^{-\Delta\Delta CT}$ method [28]. Results are shown as the mean (±SEM) fold changes in cytokine gene expression between the different vaccine groups.

2.7. Statistical analysis

The area under the curve (AUC) as described by Heldens et al., [29] was calculated by the trapezoidal rule and used as the metric for the repeated measures analysis of antibody levels.

IBM SPSS Statistics 21 (Armonk, New York, USA) was used to analyse the data. Repeated measures analysis of variance and post hoc testing was carried out using the Kruskal–Wallis and the Mann–Whitney *U* tests. Test of significance were carried out at α = 5% level.

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