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A comparison of antibody responses to commercial equine influenza vaccines following primary vaccination of Thoroughbred weanlings—A randomised blind study

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ABSTRACT

Many racing authorities, sales companies and equestrian bodies have mandatory vaccination policies for equine influenza (EI). The consequences of lack of vaccine efficacy include clinical disease, disruption to training programmes, the cancellation of equestrian events and the introduction of virus to susceptible populations. The correlation between antibody against the virus haemagglutinin and protection against influenza has been well established. The objective of this study was to compare the antibody responses of 66 unvaccinated Thoroughbred weanlings on four different stud farms, following primary vaccination (V1, V2 and V3) with the five EI vaccines commercially available in Ireland (Duvaxyn IET Plus, Equilis Resequin, Equip FT, Equilis Prequenza Te, ProteqFlu Te). Antibody responses were monitored for 6 months post V3 by single radial haemolysis. The pattern of antibody response was similar for all vaccines and for all antigens tested. A rapid decline of antibody level was observed by 3 months post V2 for all vaccines. The antibody response of the horses vaccinated with the whole virus vaccine Duvaxyn IET Plus was significantly higher than that of the horses vaccinated with the other four products. Five weanlings had maternally derived antibodies (MDA) at the time of V1. The canary pox recombinant vaccine, subunit vaccine and whole virus inactivated vaccines administered to these weanlings did not induce a detectable antibody response against the background of MDA but effectively primed the animals as revaccination resulted in a strong antibody response. In this study 43% of the weanlings failed to seroconvert after V1. This high incidence of poor responders has not been reported in previous experimental studies relating to these products. The poor responders were observed in all vaccine groups except those vaccinated with Duvaxyn IET Plus. Post V2 the incidence of poor responders was reduced to 7% and all horses responded to V3. The study demonstrates that independent evaluation of influenza vaccine performance in the field is critical to add to the body of knowledge gained from experimental challenge experiments carried out for regulatory or marketing purposes.

1. Introduction

Equine influenza virus (EIV), an orthomyxovirus, is a highly contagious respiratory pathogen of horses and other equidae. In countries where equine influenza (EI) is endemic the economic losses associated with outbreaks are minimised by vaccination. The majority of the vaccines contain representatives of the only two subtypes of influenza that are known to have adapted successfully to equidae, i.e. H3N8 and H7N7. However, H7N7 viruses have not been isolated for over two decades and the World Organisation for Animal Health or OIE stipulates that there is no longer a requirement for a representative of this subtype in EI vaccines [1]. An effective vaccine should prevent disease and virus shedding, i.e. induce both clinical and virological protection. Protection against virus shedding has been shown to correlate with the degree of antigenic relatedness of the vaccine strain to the challenge virus [2]. Mismatch between vaccine and infecting strains significantly increases the risk of an outbreak at the population level [3]. The consequences of lack of vaccine efficacy include clinical disease, disruption to training programmes, the cancellation of equestrian events and the introduction of virus to susceptible populations.

Vaccine efficacy is of importance to all countries irrespective of their disease status. Endemic countries rely on vaccination to minimise the incidence of disease and dissemination of viruses at competitions and other equestrian events. Vaccination failure at competitions has led not only to respiratory disease at

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show grounds but also to the wide geographical spread of virus when the competition is over and the horses return to their farms. Non-endemic countries rely on vaccination of imported horses, in addition to quarantine, to prevent an incursion of the virus [4]. Major outbreaks have occurred as a result of the international movement of breeding and competition horses vaccinated with vaccines that seem to have induced clinical but not virological protection, i.e. transmission occurred as a result of subclinically infected vaccinated horses shedding virus. There is epidemiological evidence that influenza virus was introduced into South Africa (1986 and 2003), India (1987), Hong Kong (1992) and Australia (2007) with vaccinated horses from North America, Europe and Japan [5–9]. Some of these incursions had devastating financial consequences for example, the control and eventual elimination of EI from Australia in 2007 is estimated to have cost one billion Australian dollars [9]. Many racing authorities, sales companies and equestrian bodies have mandatory vaccination policies that assist in ensuring business continuity. In 1981 the Turf Club in Ireland with the racing authorities in the United Kingdom and France, initiated mandatory vaccination following an epizootic of EI in Europe and North America that significantly impacted on the racing industry with reduced fields (number of horses entered for races) and cancelled meetings. The Irish Turf Club requires that race horses receive two primary vaccinations administered 21–92 days apart followed by a third vaccination administered 150–215 days after the second dose and annual vaccination thereafter [10]. All horses participating in Federation Equestre Internationale (FEI) competitions must receive an initial primary course of three doses of vaccine, a minimum of annual vaccination and a booster dose within 6 months + 21 days of competition [11]. The 21 day interval is provided to enable vaccination requirements to fit with the competition schedule.

As a result of mandatory vaccination programmes, trainers and owners represent a captive market for EI vaccines and need to be able to make informed decisions in relation to the product they use. The majority of vaccine evaluations are carried out in experimental ponies or occasionally horses, by vaccine companies or at the request of vaccine companies for submission to the regulatory authorities. Such studies tend to be conducted under optimal conditions for vaccination. It is desirable to establish that these vaccines are efficacious in the target animals in the field. The objective of this study was to carry out an independent evaluation of the immunogenicity of commercial EI vaccines in Thoroughbred horses on different premises and to provide data to veterinary surgeons who need evidence of vaccine efficacy to advise their clients about the relative benefits of different vaccines.

The immunogenicity of whole inactivated, subunit and canarypox recombinant EI vaccines can be evaluated by monitoring serological responses to the haemagglutinin (HA) [12–15]. HA is the major glycoprotein of influenza virus and is involved in attachment and entry of virus into the cell. Serum antibody against HA neutralises virus infectivity and correlates with protection [16,17]. These antibodies can be detected by haemagglutinin inhibition (HI) or by single radial haemolysis (SRH) but the latter has been demonstrated to be more reproducible between laboratories [18,19]. The correlation between SRH antibody and protection has been so well established that challenge studies to demonstrate efficacy are not required by the European Agency for the Evaluation of Medicinal Products (EMEA) for the substitution or addition of a new strain to an EI vaccine [20]. Experimental challenge studies and observations in the field suggest that horses with SRH antibody levels of 85 mm^2 or greater are clinically protected against antigenically similar viruses and that those with antibody levels of 150 mm^2 or greater are virologically protected and do not shed virus after challenge [13,17,21]. Higher antibody levels may be required if the horses are vaccinated with vaccines that have not been updated with epidemiologically relevant strains [2].

In a previous study we compared the antibody response of National Hunt horses in training to booster vaccination with the six EI vaccines available in Ireland [22]. The three whole inactivated (Prevac T Pro, Intervet; Duvaxyn IET Plus, Fort Dodge; Equilis Resequin, Intervet), two subunit (Equilis Equenza T, Intervet; Equip FT, Schering Plough) and a canarypox recombinant vaccine (ProteqFlu Te, Merial) available at the time of the study were compared. There was no significant difference between antibody responses induced following booster vaccination with any of the six vaccines. In order to eliminate the confounding effect of previous exposure to EI by natural infection or vaccination, the present study examined the serological responses of immunologically naive Thoroughbred weanlings, following primary vaccination with the five EI vaccines commercially available in Ireland at the time of this study. Two Intervet vaccines, Prevac T Pro and Equilis Equenza used in the study carried out in National Hunt horses were replaced with a subunit vaccine Equilis Prequenza Te in 2007.

Vaccine failure is most commonly reported in young racehorses [21]. When horses enter the training yards it is not uncommon for the same animals to receive different influenza vaccines over time. It is crucial that the vaccine used for the primary course of three doses stimulates a robust antibody response. The SRH antibody response to each of the five vaccines was monitored following the first three doses of vaccine, which were administered in accordance with the rules of the Turf Club. The aim of this study was to determine which of the vaccines elicited the highest antibody response in a randomised study in field conditions. High antibody levels correlate with protection against clinical disease and virus shedding therefore the use of a vaccine that elicits a strong humoral response will assist in the control of EI.

2. Materials and methods

2.1. Horses

This study was carried out on a population of 66 unvaccinated Thoroughbred weanlings on four different stud farms in Ireland. The number of weanlings were 10, 11, 26 and 19 on premises one to four, respectively. The population size on each premises was dictated by reliance on the cooperation of the stud owner to supply seronegative weanlings and agree to the necessary blood sampling regime. Weanlings of both genders were included in this study and they ranged in age from 159 to 297 days with a mean age of 235 ± 3.97 SE days at the time of administering the first dose of vaccine (V1). The weanlings were all born in 2007 to mares that varied in age from 4 to 20 years of age at the time of parturition.

2.2. Vaccines

All vaccines were purchased commercially. The different adjuvant and composition of each of the five vaccines included in this study are shown in Table 1. The multivalent vaccine Equilis Resequin (Intervet) combines EI and equine herpes virus type 1 (EHV-1) and type 4 (EHV-4). In addition, Equilis Resequin was the only vaccine included in this study where a combined influenza/tetanus vaccine was not available. In the horses vaccinated with this product, tetanus toxoid (Intervet) was administered separately by the veterinary surgeon on the same days as Equilis Resequin.

2.3. Vaccination

The horses in this study were randomly allocated one of the five vaccines using the random number generator available within Microsoft Excel. The number of weanlings allocated each of the