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Effective Priming of Foals Born to Immune Dams against Influenza by a Canarypox-Vectored Recombinant Influenza H3N8 Vaccine

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Summary

A classical limitation of early life immunization is the interference by maternally derived antibodies, which are known to inhibit the immune response to modified-live and killed vaccines. Several studies have convincingly shown that even minute amounts of maternally derived antibodies against equine influenza can strongly interfere with successful vaccination of foals born to immune mares. In this study we evaluated the response of foals born to vaccinated mares to immunization with a canarypox-vectored recombinant vaccine against equine influenza virus H3N8. The recombinant vaccine was able to efficiently prime foals in the presence of maternally derived immunity against influenza as was evidenced by a clear anamnestic antibody response when a secondary vaccination with the same vaccine was performed. The canarypox-vectored recombinant influenza vaccine therefore offers a unique opportunity to overcome the limitations of early life vaccination in the face of maternally derived immunity in foals.

Keywords: maternal antibody; canarypox-vectored vaccine; equine influenza; foal

Introduction

Early life immunization is faced with a number of challenges related to the immaturity of the neonatal immune system, induction of short-lived and often biased immune responses and the presence of maternally derived antibodies (MDA) (Siegrist *et al.*, 1998; Morein *et al.*, 2002). Unlike primates, there is no trans-placental transfer of immunoglobulins in the horse and the foal is essentially devoid of circulating antibodies at birth. During the first 24–48 h after birth the foal must ingest colostrum enriched in specific immunoglobulins. Maternal antibodies have become the basis for vaccination strategies for the protection of foals against equine influenza, an economically important respiratory disease in horses caused by influenza A virus of the H3N8 subtype (Daly *et al.*, 2004). While passively derived antibodies are usually adequate for immediate protection, the antibodies soon disappear as a result of natural decay, leaving the foal unprotected. Conflicting reports have been published on the persistence of MDA against influenza in newborn foals. In one study (Liu, *et al.*, 1985), over 50 per cent of the foals born to vaccinated mares were considered susceptible to influenza at 1 month of age. In contrast, van Oirschot *et al.* (1991), van Maanen *et al.* (1992) and Cullinane *et al.* (2001) have reported that maternally-derived antibodies against influenza persist for at least 3–6 months. Differences between the concentrations of MDA at 48 h of age most probably account for these contradictory results (van Maanen *et al.*, 1992).

The uptake of MDA is a two-edged sword. On the one hand, maternal antibodies play an essential role in the early life protection, but on the other hand

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even small amounts of passively acquired antibodies may interfere with successful immunization with live and inactivated vaccines, resulting in a window of susceptibility to infectious diseases (Morein *et al.*, 2002). For equine influenza, it is generally accepted that foals should not be vaccinated with killed influenza vaccines until MDA have decayed to a sufficiently low level (van Oirschot *et al.*, 1991; van Maanen *et al.*, 1992; Cullinane *et al.*, 2001). The earliest age at which foals may mount an immune response against influenza can be calculated from the maternal antibody titres at 1 week of age and the biological half-lives of the antibodies (van Maanen *et al.*, 1992). However, under field conditions this is impractical and as a rule of thumb it is recommended that foals from immunized mares should not be vaccinated with inactivated influenza vaccines prior to 6 months of age (van Oirschot *et al.*, 1991; van Maanen *et al.*, 1992; Cullinane *et al.*, 2001).

Consequently, new antigen presentation systems that can stimulate active immunity in horses in the presence of MDA would offer a substantial advantage over conventional vaccines. We have recently developed a modified-live recombinant influenza vaccine containing two canarypox viruses expressing the genes encoding the haemagglutinin (HA) of two epidemiologically relevant influenza A/equi-2 (H3N8) strains (Edlund Toulemonde *et al.*, 2005; Minke *et al.*, 2007). In this study, we have investigated the extent to which maternal antibodies against influenza interfere with the immune response to this vaccine.

Materials and Methods

Vaccine

A commercial serial of ProteqFlu® vaccine (Merial, Lyon, France), which contains two modified-live canarypox virus recombinants expressing the HA of the influenza H3N8 strains A/eq/Newmarket/2/93 (vCP1533) and A/eq/Kentucky/94 (vCP1529) was used. The vaccine was reconstituted with one dose (1 ml) of diluent containing Carbomer 974P (BF Goodrich Chemicals Europe NV, Belgium) just before use.

Animals

Twenty-three foals (7 Welsh mountain ponies and 16 mixed breed horses) of both sexes were used in this study. Twenty foals were born to mares that had been regularly vaccinated against influenza using recombinant and killed influenza vaccines and three foals were born to mares with no history of previous vaccination against influenza. All foals were born and kept at a farm designated for this type of work for the duration of the study, in accordance with the animal use and care guidelines of Merial. After birth all dams suckled their foals and gradually switched to a diet of hay and proprietary horse nuts. Drinking water was available *ad libitum*. Foals were identified by a microchip placed in the neck.

Experimental Design

The twenty foals born to vaccinated mares were randomly assigned to one of two groups (Group A and B) each containing 10 foals. Randomization was based on breed and the concentration of antibodies against influenza A/eq/Kildare/92 determined by single radial haemolysis (SRH) 35 days before start of the study. The three foals without maternal antibodies against influenza formed Group C. Foals from Group A received one dose of the recombinant vaccine in the presence of MDA on day 0 of the experiment. Foals were between 10 and 20 weeks of age at the time of priming. Once MDA had declined to undetectable levels (at day 170), foals from Group A and B received two doses of the recombinant vaccine administered 35 days apart. Foals from Group C were not vaccinated and served as controls to monitor field infections. All vaccinations were administered by deep intramuscular injection in the neck. The experimental design is shown in Table 1.

Serology

Blood samples were collected from a jugular vein of each foal at 1 and 4 weeks after birth and then at 2–6 week intervals. Sera were assayed by the Irish Equine Centre for antibody by single radial haemolysis (Minke *et al.*, 2007) with influenza A/eq/Kildare/92 (closely related to A/eq/Kentucky/94) as representative antigen. The titres were expressed as the area of haemolysis (mm^2).

Table 1
Experimental design

Group	*n* =	*Priming dose (day 0)*	*Booster doses (days 170–205)*	*Mean (range) age in wks on day 0*	*SRH titre (mm^2) on day 0*
A	8*	Yes	Yes	15.1 (10–20)	59.7 (33.6–89.7)
B	10	No	Yes	15.6 (12–20)	51.5 (0–118.7)
C	3	No	No	20.7 (17–25)	0

*Two foals were excluded from the study due to absence of MDA at the time of priming.