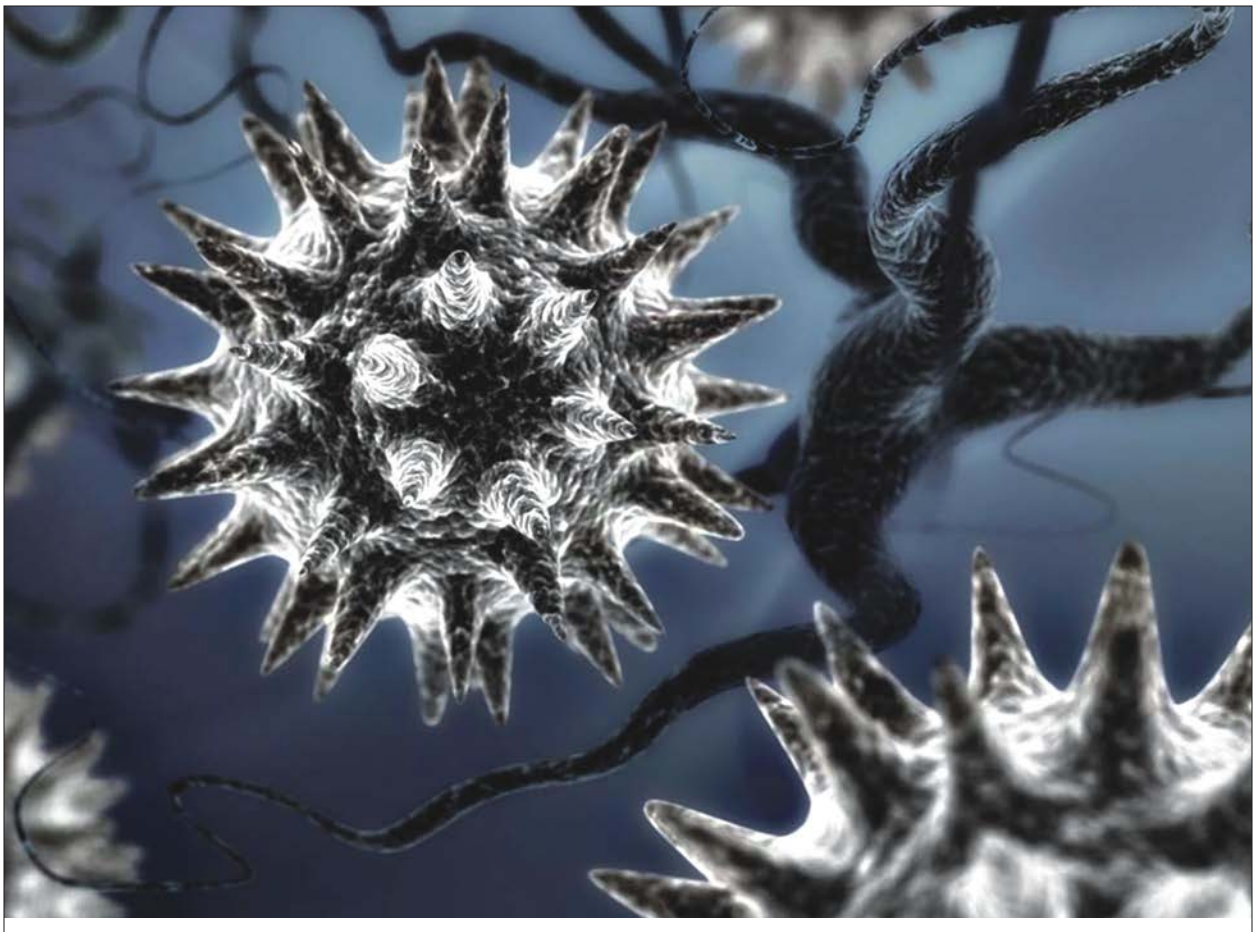

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Immune Response of Pregnant Mares and their Foals for Inactivated Equine Influenza Vaccine

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ABSTRACT

This study comprises the immune response of pregnant mares and their foals to monovalent inactivated freeze-dried Equine Influenza (EI) vaccine, reconstituted in Diethylaminoethyl (DEAE) Dextran solution as an adjuvant. It was found that mares developed high Haemagglutinating Inhibition (HI) titre at 3 weeks post vaccination and remain with protective level up to 6 months while their foals had moderate (HI) antibodies within 48 h post colostral suckling, the HI antibodies rise to a level similar to their dams within one to two weeks then persisted with considerable level up to 6 months of their age.

Key words: Pregnant mares, equine influenza (EI) vaccine, haemagglutinating inhibition (HI)

INTRODUCTION

Infection with influenza A-equi-2 virus is a common cause of rapidly spreading outbreak of respiratory diseases.

Although, horses of all ages are susceptible but young and racing horses appear to be at the greatest risk of acquiring infection.

Foal immunization is one component of an infectious diseases prevention program and absorption of maternal antibodies from colostrum is important for protection of foals against specific infectious diseases but unsuitable time of vaccination interfere with the response of foals to many vaccinal antigens.

Equine Influenza (EI) is one of the diseases of high risk for wealings and young foals, also it is an important predisposing factor to the high incidence of pneumonia in foals (Wilson, 1999). So, ingestion of adequate colostrum in the first hours of life is necessary for foals. The value of passive transfer can be considerably influenced by vaccination of mares before foaling to maximize colostral concentration of antibodies.

Foal vaccination should be started after the risk of maternal antibody interference is no longer present but it remain a continuous issue over what products should be used and when.

Thus, the present study was planned as a trial for timing the initial series of foal vaccination against Equine Influenza (EI).

MATERIALS AND METHODS

Virus: Locally identified EI virus (A/equi-2/Alex-1/08) EP6 Haemagglutinating (HA) titre 2048 and infectivity titre 9.5 $\log_{10}$ EID_{50}/0.1 mL was used for vaccine preparation.

Antisera: Reference antisera against (A/equi-1/Paraque/56) H7N7 and (A/equi-2/Miami/63) H3N8 were obtained from National Veterinary Services laboratories, United States Department of Agriculture, Veterinary Services (NVSL, USDA, VS).

Animals: Six apparently healthy pregnant mares (3-4 years old), four of them were used to study the immune response of the prepared vaccine and follow up the maternal immunity in their foals and two for safety test.

Two groups of Guinea pigs 250-300 g/weight (5/group) one of them was used to determine the potency and safety of the prepared vaccine and the other group used as a control.

Embryonated Chicken Eggs (ECE): Groups of Specific Pathogen Free (SPF) 9-11 days old ECE were used for virus propagation, egg infectivity titration and to detect the residual infective virus in the inactivated virus fluid.

Binary Ethyleneimine (BEI): The 0.1 M binary ethyleneimine was dissolved in 100 mL of 0.2 M NaOH solution (Aldrich Chemical Co. LTD) and used as virus inactivator according to Bahnemann (1990) and Hassanain (1992).

DEAE-dextran solution: Solution of Diethylaminoethyl (DEAE) Dextran solution (chloride form) was obtained from MP Biomedical LLC and used as diluent of the freeze-dried vaccine.

Identity test: The identity of EI virus fluid (A/equi-2/Alex-1/08) EP6 was confirmed by Haemagglutination Inhibition (HI) test with reference antisera against influenza virus subtype-1 and subtype-2.

Virus inactivation: According to Eman (2005) vaccine virus fluid of EI subtype-2 (EP6) with Haemagglutination (HA) titre 2048 and infectivity titre 9.5 $\log_{10}$ EID_{50}/0.1 mL was incubated with BEI in a final concentration (0.003 M) at 37°C for 24 h.

Residual infective virus activity in ECE: It was performed according to the method described by OIE (2008).

Sterility test: According to OIE (2008), preparation of freeze-dried vaccine: It was performed according to Eman (2005).

Addition of adjuvant: Each vial of the inactivated freeze-dried vaccine was reconstituted in 3 mL DEAE-Dextran solution (one vial/3mL/dose/horse). The dose must have HA titre not less than 2^8 expressed in $\log_2$.

Safety test of the locally prepared EI inactivated vaccine in horse: It was performed according to OIE (2008).

Immunogenic potency of the locally prepared EI inactivated vaccine in Guinea pigs: It was performed according to OIE (2008).

Immune response of pregnant mares vaccinated with the locally prepared EI inactivated vaccine and their foals:

- Four pregnant mares, each inoculated before foaling by one to two months I/M with the prepared EI vaccine (One vial/3mL/dose/horse), then they received a booster dose of the vaccine 4 weeks later to maximize uniformity of passive transfer
- Serum samples were collected from vaccinated mares at regular intervals
- Serum samples were collected from foals of vaccinated mares after 48, one and two weeks post partum
- Serum samples were collected from foals and their dams nearly every month up to 6 months after birth

RESULTS AND DISCUSSION

EI virus is a leading cause of respiratory diseases in horses (Paillot *et al*., 2006) and it may cause severe viral pneumonia in young foals leading to death within 48 h (Miller, 1965).

Passive transfer of antibodies via colostrum is important for protection of foals against pathogens which they may encounter during the first few months post partum (David *et al*., 2001). So, this study will try to date vaccination time of pregnant mares and their foals.

Results of EI virus titration (A/equi-2/Alex-1/08) which used for vaccine preparation was represented in Table 1 where the infectivity titre was 9.5 $\log_{10}$ EID_{50}/0.1 mL and the haemagglutinating titre was 2048 HA units.

Concerning with potency test which was performed on two groups of Guinea pigs (Table 2) group (A) inoculated with the prepared freeze-dried EI vaccine reconstituted in DEAE Dextran solution (horse dose) and group (B) used as a control.

Serum samples of group (A) (at 21 days post inoculation) showed mean HI antibody titre 921.6 while group (B) showed negative results, where the protective titre should not less than (64) according to Joseph *et al*. (1969) and OIE (2008).

Table 1: Titration of EI virus fluid.

	HA Titre *	Infectivity titre**
Influenza A/equi-2	2048	9.5*

*HA titre expressed as the reciprocal of virus dilution, **Infectivity titre expressed as $\log_{10}$ EID_{50}/0.1 mL

Table 2: HI antibody titre in sera of G. pigs inoculated with freeze-dried EI vaccine

	HI antibody titre			
	Group (A)		Group (B)	
Animal No.	Pre-inoculation	21 days post inoculation	Pre-inoculation	21 days post inoculation
1	-	1024	-	-
2	-	512	-	-
3	-	512	-	-
4	-	2048	-	-
5	-	512	-	-
Mean	-	921.6	-	-

Group (A): G: Pigs inoculated with freeze-dried EI vaccine, reconstituted in DEAE-Dextran solution (horse dose), Group (B): Control group (-): Negative result, *: No. of animal in each group

Table 3: HI antibody titre in sera of pregnant mares vaccinated with monovalent EI inactivated vaccine and the levels of maternal antibodies in their foals

	*HI antibody titre in sera of pregnant mares						*HI antibody levels in sera of foals				
Date of sample	M 1	M2	M 3	M4	Mean	Age of foals	F1	F2	F3	F4	Mean
Pre-vaccination	-	-	4	2	1.5	48 h	32	16	32	16	24
2 weeks post vacc.	8	16	16	4	11	One week	128	256	128	256	192
3 weeks post vacc.	128	256	256	64	176	2 week	256	512	256	256	320
One month post vacc.	64	128	128	64	144	One month	512	512	256	512	448
Two month post vacc.	512	512	1024	512	640	1.5 months	256	512	128	256	288
Foaling	256	256	512	256	320	2 months	128	256	128	128	160
3 month post vacc.	512	1024	512	1024	568	2.5 months	128	256	64	128	144
4 months post vacc.	1024	1024	2048	1024	1280	3 months	128	128	64	64	96
5 months post vacc.	512	512	1024	256	576	4 months	64	64	64	32	56
6 months	128	128	512	128	224	5 months	16	16	32	16	20
7 months	32	32	128	32	56	6 months	16	8	8	8	10
8 months post vacc.	16	16	64	16	28						

B: Boostering dose, M: Mare, F: Foal, *HI antibody titre: Haemagglutination inhibition antibody titre expressed as the reciprocal of serum dilution giving complete inhibition of haemagglutination. (-): Negative result, N.B.: The permissible protective levels of HI antibody was (64)

From data obtained in Table 3 which represent the immune response of pregnant mares that vaccinated with the prepared EI vaccine and the level of maternal antibodies in their foals.

The first dose of EI vaccine was able to stimulate HI antibodies in pregnant mares at 3 weeks post vaccination with mean titre (176).

Much higher levels of HI antibodies were obtained after boostering at 2 months post vaccination with mean HI titre (640).

Serum samples which were collected from the four mares just after foaling showing drop in the level of HI antibodies with mean titre (320) but within two weeks after foaling, HI antibody level began to rise again and then decline gradually with a considerable protective level of mean titre (224) at 6 months post vaccination.

Concerning with foals, within 48 h post colostral suckling, HI antibodies began to appear in sera of foals with a mean titre (24). This was in agreement with McGuire and Crawford (1973), Galan *et al.* (1986) and Sheoran *et al.* (2000) who reported that immunoglobulins were passively transferred to foals via colostrum.

Within one to two weeks post colostral suckling, the antibody titre in sera of foals rise to a level nearly similar to those of mares at foaling where the mean value of HI antibody in sera of foals at one and two weeks after birth (192, 320), respectively. These results were documented by Van Oirschot *et al.* (1991).

With the age of foals, HI antibody titre rise until one month with a mean titre (448). After that there were gradual decline in the maternal antibody level but remain within the considerable protective titre with a mean value (144) at 2.5 months of age.

At 3 months of age, there were further decline in HI antibody titre until the age of 6 months, where only low level of antibodies were detectable with a mean HI titre (10).

From the previous results, we can conclude that maternal antibodies began to appear in sera of foals within 48 h post colostral suckling (McGuire and Crawford, 1973; Sheoran *et al.*, 2000). Antibody titres in sera of foals shortly after birth were similar to those of mares at foaling (Van Oirschot *et al.*, 1991).

In sera of foals born to vaccinated mares, maternal antibodies persisted for 3-6 months of age (Van Oirschot *et al*., 1991; Conboy *et al*., 1997; David *et al*., 2001).

From the results and conclusions, it is deduced that maternal antibody titre at the time of vaccination is closely related to the degree of interference with the immune response because even low titre of maternal antibodies might interfere with the efficacy of vaccination against EI (Van Mannen *et al*., 1992; Conboy *et al*., 1997).

So, it is recommended to vaccinate pregnant mares during last 2 months of gestation to maximize colostral concentration of antibodies and it is very important to recommend that primary immunization against EI for foals born to vaccinated mares should not commence before 6 months of age and include 3 doses in the primary series.

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