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Studies on evaluation of inactivated lyophilized Equine Influenza vaccine in Egypt

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SUMMARY

Two batches of egg adapted monovalent inactivated freeze dried equine influenza vaccine (EIV) were evaluated. There was no abnormal appearance of the freezed disc, prepared from locally isolated strains. The efficacy was tested by lab animal (Guinea pigs) and target animal (horse). The vaccine proved to be safe and potent for both guinea pigs and horses. The mean haemagglutination inhibition (HI) antibodies of the vaccine reconstituted in DEAE-Dextran solution (as a solvent and adjuvant) were 120.4 and 153.7 in G. pigs, 179.2 and 230.4 in horses for the two batches of vaccine respectively. The keeping quality of the local prepared vaccine was studied. Shelf validity was stable at 4°C for one year, could be kept at -20°C for 3 years and 10 months at 40°C.

INTRODUCTION

Equine influenza (EI) is one of the most serious viral respiratory diseases among horses of all ages with world wide spread. EI is an infectious highly contagious acute febrile disease and is abading cause of respiratory disease (Paillot et al., 2006). It is characterized by high morbidity may reach 90% (Gerber, 1970) and low mortality rate except in young foals due to sever pneumonia (Bryans, 1964).

EI is caused by virus belonged to orthomyxovirolae, therefore, two antigenically distinct subtypes (H7N7, H3N8).

outbreak in winter 1999-2000 (Hamoda et al., 2001, Nashwa, 2004). A recent outbreak occurred in 2008 (Soliman et al., 2008). The most recent outbreak in world, in Australia (2007) caused by H3N8 of an avian origin causing high mortality and morbidity.

It is clear that the last major outbreaks in last 20 years had been caused by EI subtype 2 (H3N8) which appears to be a mutation of avian influenza virus (Jan et al., 2004).

Because vaccination is one of the most powerful means of the controlling of EI disease, thus the present study was designated to evaluate locally prepared inactivated monovalent freeze-dried EI vaccine reconstituted in DEAE-Dextran.

MATERIAL AND METHODS

1. Material:

a.Seed virus:

Locally isolated strains of EI virus (A/equi-2cairo/2000) EPS with HA titre 2048 and infectivity titre 9-5 $\log_{10}$ EID_{50}/0.1 ml was used for vaccine preparation.

b. Vaccine:

Monovalent freeze-dried equine influenza vaccine produced by Veterinary Serum and Vaccine Research Institute, Abbasia (VSVRI), Cairo. The dose must contain HA titre not less than 2^8 expressed in $\log_2$.

c. Antisera:

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

d. Animals:

Four groups of apparently healthy susceptible horses 2-4 years old and groups of G. pigs 260-300 gm were used to evaluate the potency, safety and stability of the prepared vaccine.

2. Methods:

a.1. Identity test:

It was confirmed by HI test using reference monovalent antisera against EI subtype-1 and 2.

a.2. PCR:

It was done according to Donofrio et al. (1994), Nashwa and Noha (2008).

b.1. Completion of inactivation test:

It was carried out via allantoic inoculation of embryonated chicken egg (ECE) according to **OIE (2008).**

b.2. Sterility test:

Samples of inactivated vaccine were cultured for detection of aerobic, anaerobic bacteria, mycoplasma and fungal contamination according to **Code Federal Regulation (2005).**

contamination according to **Code Federal Regulation (2005).**

3. Safety test:

I/M injection of 2 pregnant mares with one dose of freeze-dried EI vaccine reconstituted in DEAE-Dextran solution followed by another dose injected after 4 weeks (OIE, 2004) and kept under observation for 10 days after the second dose.

4. Potency test:

a. In Guinea pigs:

According to OIE (2004), five seronegative G. pigs were injected S/C at two different sites with horse dose (3 ml) of the reconstituted tested vaccine and another group was kept as (–ve) control at the same condition. Serum samples were collected from each group 3 weeks later, antibody immune response were assayed by HI test.

b. In horses:

Five susceptible seronegative horses for EI were I/M injected with EI freeze-dried vaccine reconstituted in DEAE-Dextran (3 ml /dose/horse). Four weeks later of booster dose of the same vaccine was injected. Three serum samples was collected as follows, the first blood sample at the time of vaccination, the second sample one week after the first dose and the third sample 2 weeks after the booster vaccination. All serum samples were screened for the immune response using HI test.

5. Keeping quality of the tested vaccine:

Random samples of the vaccine were kept at different temperatures; 4°C, room temperature 25-30°, 40°C and -20°C for 36 months. Testig the samples every 2 weeks by inoculation of G. pigs, their serum samples were testing for efficacy using HI test.

RESULTS AND DISCUSSION

Equine influenza (EI) is one of the important diseases causing respiratory manifestation causing high morbidity and mortality specially in young foals. So, vaccination is the main goal of controlling this disease.

The major difficulty facing the immunizing power of the prepared vaccine is the antigenic variation viral strains with periodic minor antigenic changes in haemagglutinin (HA) and neuraminidase (N) (antigenic drift), is the major factor behind in yearly the continuous occurrence of EI (Kumar, 2007).

The identity test proved to be positive for H3N8 EI virus by RT-PCR (Fig. 1).

Concerning the safety of inactivated freeze-dried vaccine reconstituted in DEAE-Dextran solution, no abnormal clinical of inoculated pregnant mares either at site of

On the other hand, tables (3, 4) and Fig. (2, 3) show that the mean HA antibodies titre in horse sera (6 weeks post vaccination) were 1792 and 2304 for the two vaccine batches respectively. These results agree with that of Joseph et al. (1969) and OIE (2004) who concluded that the protective HI antibody titres should be not less than 64.

From table (5), it is obvious that there are no effect of storage on the tested vaccine that kept at 4°C, 25-30°, 40°C and -20°C for 18, 12, 9 and 36 months 0n the immune response respectively. These results agree with that of Pumper and Yamashiroya (1975) and Eman (2005).

From the fore-mentioned data,It can be concluded that the evaluated monovalent inactivated freeze-dried EI vaccine succeeded to induced the desired immune response in horses. The immune response of G. pigs has a parallel patterns to that of horse so we can use G. pigs as good model to evaluate the vaccine where the protected HI titer is 64.

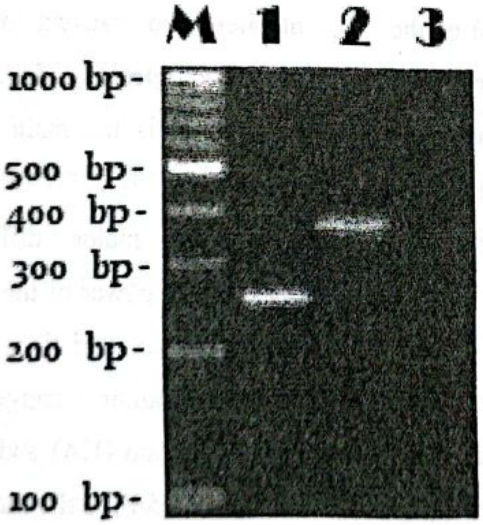


Fig. (1): RT-PCR products of equine influenza virus vaccine; the M lane represent 100 bp DNA ladder; Lane 1 represent 244 bp amplicone of the Matrix gene; Lane 2 represent 375 bp amplicone of the H3 gene; Lane 3 negative control (RNA of LaSota Virus)

Table (1): Safety test in pregnant mares inoculated with inactivated freeze dried equine influenza (EI) vaccine reconstituted in DEAE - Dextran solution

Days post first inoculation	Recorded temperature (°C)			
	Batch (1)		Batch (2)	
	Mare 1	Mare 2	Mare 3	Mare 4
1	37.4	37.6	37.5	37.5
2	37.4	37.5	37.5	37.5
3	37.5	37.6	37.6	37.6
4	37.5	37.6	37.5	37.6
5	37.5	37.6	37.6	37.6
6	37.4	37.8	37.5	37.6
7	37.4	37.6	37.6	37.5
8	37.5	37.6	37.6	37.6
9	37.5	37.5	37.5	37.6
10	37.5	37.6	37.5	37.6
Boostering				
1	37.5	37.5	37.5	37.5
2	37.5	37.5	37.5	37.6
3	37.6	37.4	37.5	37.5
4	37.5	37.5	37.5	37.6
5	37.5	37.6	37.5	37.5
6	37.5	37.7	37.5	37.6
7	37.5	37.6	37.6	37.5
8	37.5	37.6	37.5	37.5
9	37.5	37.6	37.5	37.6
10	37.5	37.5	37.6	37.5

Table (2): HI antibodies titer in Guinea pigs sera inoculated with 2 batches of the prepared vaccine

Animal number	HI antibodies titer				
	Batch (I)		Batch (II)		control
	Pre vaccination	21 dpv	Pre vaccination	21 dpv	
G1	-	128	-	128	-
G2	-	128	-	128	-
G3	-	256	-	128	-
G4	-	128	-	256	-
G5	-	64	-	128	-
Mean	-	120.4	-	153.7	-

* dpv: days post vaccination
* HI titer expressed as a reciprocal of virus

Table (3): HI antibodies titer in horses sera inoculated with batch I of EI vaccine

Time of sampling	HI antibodies titer in horse sera*							
	Batch I of the EI vaccine						control	
	H1	H2	H3	H4	H5	mean	H1	H2
Pre-vaccination	8	8	8	8	4	7.2	8	4
2 wpv	32	16	16	32	16	22.4	8	4
3 wpv	32	64	32	32	16	33.2	8	4
4 wpv	32	64	32	32	32	38.4	8	4
Boostering								
6 wpv	32	128	128	256	128	179.2	4	4
8 wpv	64	256	256	256	128	192	4	4

Table (4): HI antibodies titer in horses sera inoculated with batch II of EI vaccine

Time of sampling	HI antibodies titer in horse sera							
	Batch II of the EI vaccine						control	
	H1	H2	H3	H4	H5	mean	H1	H2
prevaccination	8	8	4	4	8	6.4	4	4
2 wpv	32	32	16	16	32	25.6	4	4
3 wpv	64	64	64	32	128	70.4	4	2
4 wpv	128	128	64	128	128	151.2	4	2
Boostering								
6 wpv	256	256	128	256	256	230.4	2	2
8 wpv	256	512	256	256	256	307.2	2	2

* HI antibodies titer was expressed as a reciprocal of virus dilution

Table (5): Keeping quality of the prepared vaccine

Storage Temp. (°C)	G. pig group (5 G. pig /groups)	Time of storage	Mean HI antibody titer of G. pigs sera vaccine	
			vaccinated	control
4	A	0 time	140	-ve
		3 month	140.8	
		6 month	102.4	
		12 month	102.4	
		18 month	16	
25-30	B	0 time	140	-ve
		3 month	64.8	
		6 month	16	
		12 month	-ve	
40	C	0 time	-ve	-ve
		3 month	-ve	
		9 month	-ve	
-20	D	0 time	140	-ve
		3 month	140	
		6 month	140	
		12 month	140	
		24 month	102.4	
		30 month	95.8	
		36 month	92.4	

* Mean HI titer expressed as a reciprocal of serum dilution giving complete inhibition of heamagglutination
* -ve: Negative

Fig. (2): HI antibodies titer in horses sera inoculated with batch I of EI vaccine

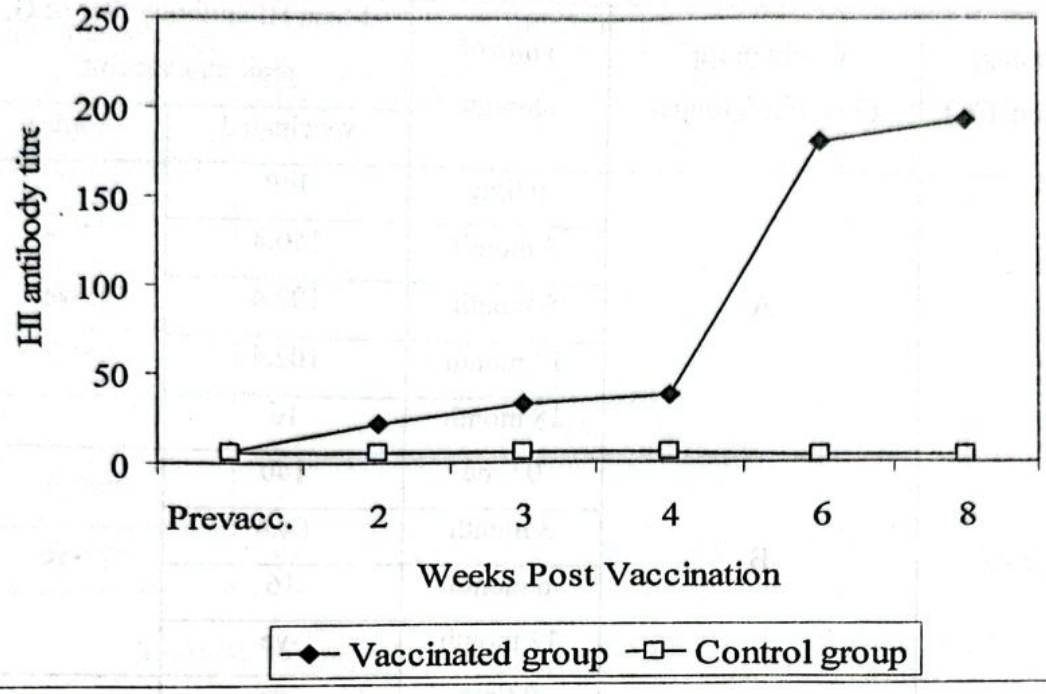
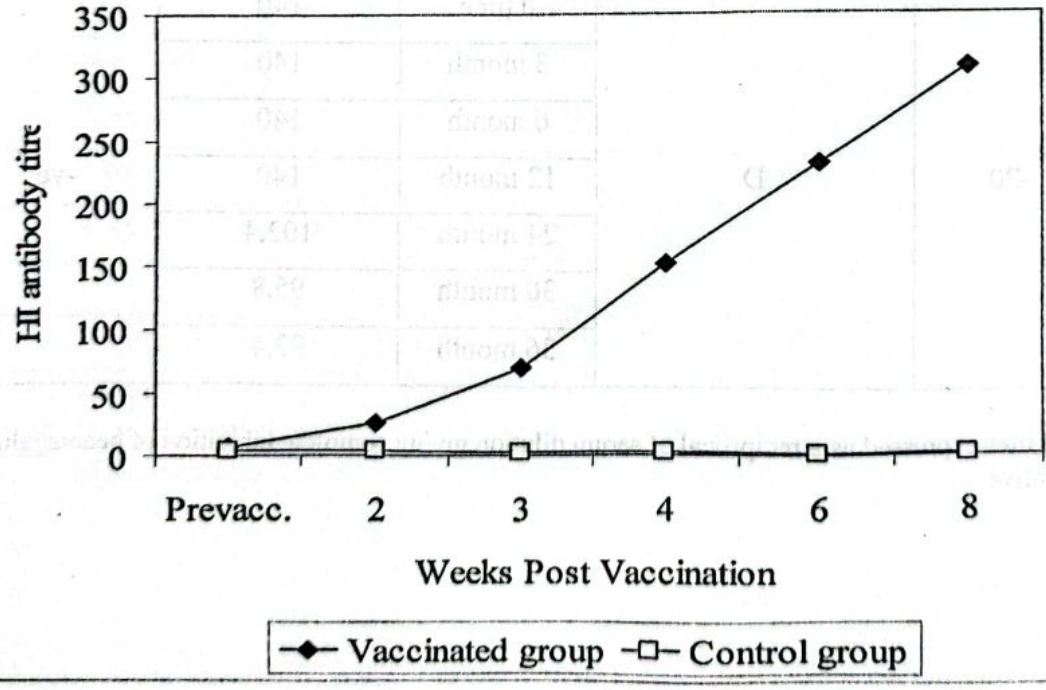


Fig. (3): HI antibodies titer in horses sera inoculated with batch II of EI vaccine



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دراسات على معايرة لقاح انفلونزا الخيول المجفد فى مصر

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• المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية العباسية القاهرة
•• معهد بحوث الأمصال واللقاحات البيطرية العباسية القاهرة

تم تقييم دفعتين من لقاح إنفلونزا الخيول إحادى العترة المثبط المجفد لا يوجد ظواهر غير طبيعيـــة للقاح باستخدام اختبار التلازن الدموى المثبط كان 1254، 1537 فى خنازير غينيـــا و 1792، 2354 فـــى الخيول للدفعتين على التوالى.

تم دراسة درجة كفاءة اللقاح المحلى المحضر وكفائته. كان اللقاح ثابت عند درجة حرارة 4°م لمـــدة عام ويمكن حفظه عند درجة -20°م لمدة ثلاث لأعوام وحفظه ايضاً لمدة 10 شهور عند درجـــة حـــرارة 40°م.