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IMMUNE RESPONSE IN HORSES VACCINATED WITH A COMBINED FREEZE DRIED VACCINE AND TETANUS TOXOID

(With 5 Tables)

By

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دراسة الاستجابة المناعية فى الخيول المحصنة باللقاح الجامع المجفف لفيروس طاعون الخيل متعدد العترات المثبط وتوكسويد التيتانوس

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أقيمت دراسة معملية لتقييم لقاح طاعون الخيل المتعدد العترات (٦، ٧، ٨) المثبط والمجفف بعيارية قدرها ٥ر٧ ، ٥ر٦ ، ٥ر٧ لو ١٠ جرعة نصف معدية لكل ١مل على التوالى. وقد تم تثبيط هذه العترات الثلاث كل على حده باضافة البينارى اثيلين أمين عند درجة حرارة ٣٧ °م وتركيز جزيئ ٠٠٤ر لمدة ٢٤ ساعة. ولتقييم القوة المناعية للقاح المحضر تم عمل التجارب الأولية على ٦ خيول مقسمة الى ثلاث مجموعات (٢ حصان/مجموعة). تم حقن المجموعة الاولى عضليا باللقاح المتعدد العترات (٦، ٧، ٨) المجفف والمثبط بواسطة البينارى اثيلين أمين والمتحد بتوكسويد التيتانوس النقى والذى أذيب فى محلول دايا- ديكستران (١٠٠ مجم/مل). أما المجموعة الثانية فقد تم حقنها عضليا بنفس اللقاح المثبط ولكن دون اضافة توكسويد التيتانوس. وبالنسبة للمجموعة الثالثة فقد تم حقنها تحت الجلد باللقاح المتعدد العترات (٦، ٧، ٨) والمثبط والمجفف ولكن أذيب فى محلول الملح الفسيولوجى المعقم. وقد قيمت الاستجابة المناعية للخيول المختبرة بواسطة الاختبارات الاتية: السيرم المتعادل، الاليزا والمثبت المكمل. يتضح مما سبق ان المجموعة الثالثة من الخيول المحقونة أعطت استجابة مناعية ضعيفة حتى بعد الحقن بالجرعة المنشطة. أما المجموعة الأولى والثانية من الخيول المحقونة أعطت استجابة مناعية عالية وسريعة للجرعة المنشطة وكذلك بعد اعادة التحصين.

SUMMARY

The present study was devoted to evaluate the immunogenic potency of three different types of inactivated freeze dried vaccines of African Horse Sickness (AHSV). Each of the three vaccines was comprised of the three serotypes of AHSV (6, 7 and 8) but with a titre of 7.5, 6.5 and 7.5 $\log_{10}$ $TCID_{50}$/ ml, respectively. The viruses were inactivated separately at 37°C by addition of binary ethyleneimine (BEI) at a final concentration of 0.004 m for 24 hours. The study was conducted on six adult susceptible horses which were divided into 3 groups (2 horses/ group). The first group (a) was inoculated intramuscularly (I/M) with the first type of vaccine combined with a purified tetanus toxoid (100 LF/3 ml) freeze dried vaccine, reconstituted in DEAE-dextran (100 mg/ml) solution. The second group (B) was inoculated I/M with the second type after reconstitution in DEAE-dextran solution. The third group (C) was inoculated subcutaneously with the third type after reconstitution in sterile saline solution. The immune response of the horses was evaluated using serum neutralization test (SNT) and complement fixation test (CFT). The third type was weak antigenically and unapplicable inducing a low and short seroconversion antibody response after primary and booster doses. Both the first and second type induced a rapid and high seroconversion after boostering and re-vaccination.

Key words: *Horses-Immune Response-Vaccine- Tetanus Toxoid.*

INTRODUCTION

African Horse Sickness (AHS) is a disease with a well defined geographical distribution, and equally well defined seasonal occurrence. The disease is transmitted by blood suckling arthropods; culicoides and mosquitoes are highly suspected (Dutoit, 1944, Ozawa et al., 1966b). Several epizootics of AHS occurred in Egypt. The first epizootic of AHS was recorded in 1928 (Carpano, 1930), followed by two successive epizootics in 1943 and 1953 (Alexander, 1948; Wahby, 1958). Later on, a minor epizootic of AHS was reported by Farid et al. (1981), in Aswan during 1971, the isolates were identified as AHS virus serotype 9. Also, AHS epizootic was reported in Saudi Arabia in 1989 where AHS virus serotype 9 was identified (Anderson et al., 1990).

AHS killed virus vaccine was used even before live attenuated vaccine. McIntosh (1958), Dutoit and Alexander (1930) and Walker (1941) prepared

inactivated horsesickness vaccine by adding formalin to infected horse emulsion. Several workers succeeded in preparing an efficient monovalent inactivated vaccine either from the neurotropic or viscerotropic strains using different kinds on inactivation (formalin-B propiolactone-merthiolate-binary ethyleneimine) and various types of adjuvants-(Aluminium hydroxide gel, saponin, incomplete Freund's adjuvant) Mirchamsy et al., 1968; Bourdin et al., 1976; Mirchamsy et al., 1973 and Hassanin, (1983).

The present study was undertaken in order to produce an effective, safe and applicable combined polyvalent inactivated freeze dried vaccine against AHS virus to vaccinate equines which have to be shipped to horse-sickness free countries or vice versa and also for the immunization of very valuable Arabian or foreign imported horses.

MATERIAL and METHODS

1- Viruses:

Three mouse adapted neurotropic (attenuated) strains of AHS virus (MO_{103}, MS_4, VER_3) serotype 6 (114), 7 (Karen) and 8 (18/60) were used for the production of the inactivated freeze dried vaccines. These strains were propagated by intracutaneous (I/c) inoculation for 103 passages in adult mice at Onderstepoort Laboratory, South Africa. Also, For 4-5 successive passages in monkey kidney stable cell (MS) cultures at FADDL, Plum Island, USA and 3 passages in African Green Monkey Kidney (VERO) cell cultures at Vet. Serum and Vaccine Research Institute (VSVRI), Cairo. These passages were carried out for adaptation of these strains in tissue cultures.

2- Antisera:

Antisera against AHS virus, serotypes 6, 7 and 8 were prepared in goats and obtained from FADDL, Plum Island, USA. Antisera against AHS virus, serotype 9 (neurotropic, mouse brain) propagated strain (S_2) was prepared in goats at Vet. Serum and Vaccine Research Institute (VSVRI), Cairo. Anti-horse IgG (H+L) peroxidase conjugate, code pp 260, lot G410 produced by the binding site Birmingham Research Park, UK.

3- Purified antigen of AHS virus:

A purified antigen of the VERO cell cultures infected with AHS virus, serotype 9 (strain S_2) was prepared according to the method described by Salama et al. (1977).

4- Residual virus assay:

According to the method described by Stellman et al. (1970), residual virus assay in the BEI inactivated virus fluid collected 24 hours after incubation at 37°C was done in both suckling mice and VERO cell cultures.

5- Tetanus Toxoid:

An aqueous solution of purified tetanus toxoid was titrated, so that each 1 ml would contain 1000 LF.

6- Preparation of freeze dried vaccines:

The vaccine virus fluid was prepared from the 6th or 7 th virus passages in VERO cell cultures. All the serotypes 6, 7 and 8 were titrated and titres calculated according to the method described by Reed and Muench (1938).

Equal volumes of virus fluids of the three serotypes (6, 7 and 8) were pooled and inactivated with Binary ethyleneimine (BEI) at final concentration 0.004M. Virus fluid inactivator mixtures were inoculated with continuous stirring at 37°C for 24 hours followed by immediate addition of 20% sterile sodium bisulfite solution to neutralize the toxic action of residual inactivators on target host, as mentioned by Hassanin (1983).

The freeze drying procedure was done according to the method described by Ozawa et al. (1966a). Equal volumes of polyvalent BEI- inactivated freeze dried vaccine of AHS virus - serotypes 6 (114), 7 (Karen) and 8 (18/60), and tetanus toxoid diluted in lactose peptone tris buffer (LPTB) (200 LF/3 ml) were mixed and dispensed in sterile vials (3 ml/ vial, followed by freeze drying in Edward's apparatus for 24 hours. The prepared vaccines were stored at- 20°C till used.

7- Potency test of polyvalent BEI inactivated freeze dried vaccines of AHS virus seotypes 6, 7 and 8 in horses:

The potency test of vaccines was determined by studying the kinetics of antibody formation in two groups of six adult horses (4-6 years old). Group (A) of horses No. 1 and 2 was each inoculated with polyvalent BEI-inactivated AHS virus- serotypes 6, 7 and 8- tetanus toxoid combined freeze dried vaccine reconstituted in DEAE-Dextran solution (one vial/ 3ml/ dose/ horse). Group (B) of horses No. 3, 4 was each I/M inoculated with polyvalent BEI-inactivated freeze dried vaccine of AHS virus- serotypes 6, 7 and 8 reconstituted in DEAE-Dextran solution. Group (C) of horses No. 5, 6 was each inoculated subcutaneously (S/C) with BEI- inactivated freeze dried vaccine reconstituted in sterile saline solution. Boostering dose of the same vaccine was injected in horses of groups (A) and (B) at the 6 th week after the primary dose and in horses of group (C) at the 4 th week. Blood samples were collected every week until one month then every two weeks up to the 6

th month post vaccination. Horses No. 1,2 (Group A) and No. 3, 4 (Group B) were re-vaccinated at the 6 th month after primary dose and blood samples were collected every month up to the 6 th month after re-vaccination. Sera were collected and frozen at- 20°C, till tested by SNT, ELISA and CFT. Also, tetanus toxoid antibodies in sera of vaccinated horses were titrated using ELISA.

8- Serological techniques:

The collected sera samples were subjectd to serological investigation using the following tests:

1- Complement fixation test (CFT) was applied according to the method described by House et al. (1990).

2- Enzyme linked immunosorbent assay (ELISA), the procedure was dose as mentioned by William (1987) and House et al. (1990).

3-Serum neutralization test (SNT) was performed according to Parker (1974).

RESULTS

Tables (1, 2, 3 and 4) show the results detecting AHS viral antibodies in sera of horses vaccinated with polyvalent BEI inactivated freeze dried vaccines against AHS virus serotype 6, 7 and 8, reconstituted either in sterile saline solution or in DEAE-Dextran solution or combined with the tetanus toxoid, then reconstituted in DEAE-Dextran solution.

Meanwhile, Table (5) shows the results of seroconversion to tetanus toxoid in horses inoculated with polyvalent BEI-inactivated AHS virus-serotypes 6, 7 and 8, and tetanus toxoid in a combined freeze dried vaccine.

DISCUSSION

The relatively recent records of AHS in South of Spain Portugal and Morocco between 1987 and 1989 as well as in Saudi Arabia in 1989 has focused attention on the need for strategy to control and prevent further spread of the disease. The establishment of large buffer zones of immunized animals between infected and free zone was recommended. OIE report has been suggesting the need of these countries for an efficient inactivated vaccines of AHS virus to substitute the present attenuated vaccines for protection of equines during epizootics (emergency vaccination and for immunization of equines in the recommended buffer zones (prophylactic vaccination).

In Egypt, from 1983 and up to 1993, the vaccination regime was confined to Aswan, Qena and Suhage. In the beginning of 1994, the Veterinary Authority (in Egypt) stopped the vaccination programme against AHS. Meanwhile, the Serum and Vaccine Research Institute started to prepare a strategic freeze dried vaccine of inactivated AHSV that can be used for further emergency vaccination of equines. In order to produce an efficient and applicable inactivated freeze dried vaccines of AHS virus, the virus titre should be not less than 6 $\log_{10}$ $TCID_{50}$/ ml (Mirchamsy and Taslimi, 1968). Therefore, the immunogenic capacity of the inactivated vaccine against AHSV, serotypes 6, 7 and 8 was evaluated. Besides, the immuno-modulating effect of tetanus toxoid on the immune response to AHSV was determined. The potency test in target animals (Horses) was used for the evaluation of these vaccines. The results of the experimental work in this study show the following:

1- The rise and fall in AHSV neutralizing antibodies was parallel with that of ELISA antibodies.

2- CF antibodies appeared earlier and declined more rapid than AHSV neutralizing and ELISA antibodies.

3- The immune response of horses in Group (B), injected with BEI-inactivated AHSV freeze dried vaccine, reconstituted in DEAE-Dextran solution, was relatively similar to that produced in horses of Group (A) injected with BEI-inactivated AHSV-tetanus toxoid combined freeze dried vaccine reconstituted in DEAE-Dextran. In Group (C), the horses immunized with BEI- inactivated AHSV freeze dried, reconstituted in sterile saline were developing low levels of AHS virus antibodies.

The immunogenic potency of a prepared freeze dried vaccine was evaluated using the indirect method of Machowiak et al. (1959), Mirchamsy and Taslimi (1968). They proved that horses immunized with Aluminium hydroxide adjuvant vaccine of either formalin or beta propiolactone inactivated AHS virus, serotype 9 developed a neutralizing antibody level at the 6 th month after boostering with titres (4, 8, 16, 32, 64 and 128) were resistant to challenge with rise of temperature not exceeding 40.7 with no other signs. Also, Bourdin et al. (1970) stated that the maximum neutralizing antibodies of NI (4) and (4.5) were recorded, respectively either in sera of horses immunized with inactivated vaccine of AHS virus serotype 9 or in sera of horses vaccinated with monovalent attenuated one at 2 nd month after vaccination which could resist the chalenge. These results represent the same obtained data in this study as depicted in Tables (1, 2 and 3).

Concerning antibody response against tetanus toxoid in horses of Group (A), it is clear that horses exhibited progressive seroconversion and produced a desirable high level of ELISA antibodies at the 2 nd week post vaccination with a mean titre of (120) and the 2 nd week after boostering with a mean titre of (1440) (Table 5).

The obtained results showed that each vaccinated horses developed anamnestic response to tetanus toxoid, as previously suggested by Scheilel (1955).

The conclusion which can be uptaken from the results of this study is the fact that both the first and second types of AHSV freeze dried vaccine are absolutely efficient and applicable.

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Table (1): Neutralizing antibodies in sera of horses experimentally vaccinated with BEI-inactivated freeze dried vaccine against AHS virus serotypes 6, 7 and 8 after 6 months after vaccinati

Time of Serum Samples collection	Results of SNT for group A			Results of SNT for group B			Results of SNT for group C		
	H1	H2	Mean Index	H3	H4	Mean Index	H5	H6	Mean Index
Pre-vacc.	--	--	-				--		
1 WPV	--	--	--				--		
2 WPV	0.75*	0.75*	0.75*	0.75*	0.75	0.75*	1.00*	1.25*	1.1*
3 WPV	1.25	2.00	1.60	0.00	1.50	1.30	0.75	1.00	0.90
4 WPV	1.50	3.00	2.30	1.25	1.75	1.50	(B)	(B)	--
(B) 6 WPV	1.50	2.25	1.90	1.75	2.25	2.00	3.00	2.00	2.50
8 WPV	4.75	5.00	4.90	33.75	4.00	3.70	2.50	1.25	2.00
10 WPV	4.25	4.50	4.40	4.00	3.75	3.70	1.75	1.25	1.50
12 WPV	4.25	4.50	4.40	4.00	3.50	3.80	1.25	1.25	1.25
14 WPV	4.00	4.00	4.00	3.75	3.25	3.50	0.75	1.00	0.90
16 WPV	4.00	4.00	4.00	3.25	3.25	3.30	--	--	--
18 WPV	4.25	3.75	4.00	2.75	2.75	2.80	--	--	--
20 WPV	4.00	4.00	4.00	2.50	2.75	2.60	--	--	--
22 WPV	4.00	3.90	4.00	2.25	2.75	2.50	--	--	--
24 WPV	3.75	3.90	3.80	2.50	2.50	2.50	--	--	--
26 WPV	3.00	3.25	3.10	2.75	1.75	2.30	--	--	--

H = Horses

B = Boostering

WPV = week post vaccination

* = Log NI50

- = Negative

Table (2): ELISA antibody titers in sera of horses vaccinated with BEI-inactivated freeze dried vaccine against AHS virus serotypes 6, 7 and 8, six months after vaccination

Time of Serum Samples collection	Results of ELISA for								
	Group (A)			Group (B)			Group (C)		
	H1	H2	Mean Index	H3	H4	Mean Index	H5	H6	Mean Index
Pre-vacc.	--	--	--	--	--	--	--	--	--
1 WPV	*10	5	7.5	--	--	--	--	--	--
2 WPV	40	20	30	10	10	10	20	20	20
3 WPV	80	80	80	40	20	30	10	20	15
4 WPV	160	100	130	80	20	50	(B)10	(B)10	10
(B) 6 WPV	40	20	30	40	20	30	40	20	30
8 WPV	1280	640	960	960	640	320	20	20	20
10 WPV	>1280	640	>960	640	640	640	20	10	15
12 WPV	1280	640	960	640	640	640	10	10	10
14 WPV	640	640	640	640	160	250	10	10	10
16 WPV	480	640	560	320	160	240	--	--	--
18 WPV	320	240	280	320	160	240	--	--	--
22 WPV	320	320	320	160	160	160	--	--	--
24 WPV	160	160	160	40	80	60	--	--	--
26 WPV	160	160	160	20	20	20	--	--	--

H = Horses

WPV = week post vaccination

B = Boostering

* = Log NI50

- = Negative

Table (3): Complement fixing antibody titers in sera of horses vaccinated with BEI-inactivated freeze dried vaccine against AHS virus serotype 6, 7 and 8, 6 month after vaccination

Time of Serum Samples collection	Results of CFT for								
	Group (A)			Group (B)			Group (C)		
	H1	H2	Mean index	H3	H4	Mean index	H5	H6	Mean index
Pre-vacc.	--	--	--	--	--	--	--	--	--
1 WPV	8	16	12	8	8	8	4	--	2
2 WPV	16	32	24	16	16	16	8	8	8
3 WPV	12	16	24	16	8	12	4	8	6
4 WPV	16	16	16	8	8	8	(B)8	4	6
(B) 6 WPV	16	8	12	16	64	40	16	16	16
8 WPV	256	128	192	64	64	64	16	8	12
10 WPV	256	128	192	64	32	48	8	4	6
12 WPV	128	64	96	32	32	32	4	4	4
14 WPV	64	64	64	64	16	40	--	--	--
16 WPV	64	32	48	32	16	24	--	--	--
18 WPV	32	32	32	16	8	12	--	--	--
20 WPV	32	16	24	8	8	8	--	--	--
22 WPV	32	32	32	8	8	8	--	--	--
24 WPV	32	16	24	8	8	8	--	--	--
26 WPV	16	16	16	8	8	8	--	--	--

H = Horses

B = Boostering

WPV = week post vaccination

- = Negative

Table (4): Cummulative of AHS antibodies against serotypes 6, 7 and 8 in sera of horses inoculated with polyvalent BEI - inactivated freeze dried vaccine, 6 months after revaccination

Time of Serum Samples collection	Neutralization indices						ELISA - antibody titers						CF - Antibody Titers					
	Group A			Group B			Group A			Group B			Group (A)			Group (B)		
	H1	H2	Mean Index	H3	H4	Mean Index	H1	H2	Mean Index	H3	H4	Mean Index	H1	H2	Mean Index	H3	H4	Mean Index
26 WPV	*3.00	3.25	3.10	2.75	1.75	2.30	160	160	160	20	20	20	16	16	16	8	8	8
1 MPRV	6.00	6.00	6.00	4.75	5.00	4.90	1280	1280	1280	640	640	640	256	128	192	64	64	64
2 MPRV	6.00	6.00	6.00	4.50	4.00	4.50	640	640	640	320	640	480	128	64	96	64	32	48
3 MPRV	5.50	5.75	5.60	4.25	4.00	4.10	640	480	560	320	320	320	64	64	64	32	32	32
4 MPRV	5.50	5.50	5.50	4.00	3.75	3.90	640	320	480	480	480	480	64	64	64	16	32	24
5 MPRV	4.75	4.50	4.60	4.00	3.25	3.60	320	320	320	240	160	200	32	32	32	16	16	16
6 MPRV	3.75	3.50	3.60	2.25	2.25	2.25	240	320	160	160	160	160	32	32	32	8	8	8

H = Horses

WPV = Week post vaccination

* = log NI50

MPRV = Month post revaccination

Table (5):

Tetanus Toxoid ELISA-antibody titers in Sera of horses vaccinated with polyvalent BEI-inactivated AHS against serotypes 6, 7 and 8, Tetanus toxoid combined freeze dried vaccine, 6 month after vaccination and 6 months after re-vaccination

Serum Time of sample Collection	Tetanus Toxoid ELISA antibody titers		
	H. 1	H.2	Mean titer
Pre vacc.	--	--	--
1 WPV	1.0*	10	10
2 WPV	80	160	120
3 WPV	40	80	60
4 WPV	40	80	60
6 WPV	40	40	40
8 WPV	>2560	320	1440
10 WPV	2560	160	1360
12 WPV	1280	160	720
14 WPV	1280	160	720
16 WPV	640	160	400
18 WPV	640	80	360
20 WPV	160	80	120
22 WPV	160	40	100
24 WPV	80	40	60
26 WPV	>2560	1280	1920
1 MPRV	>2560	1280	1920
2 MPRV	2560	640	1600
3 MPRV	1280	640	960
4 MPRV	640	320	480
5 MPRV	320	160	240
6 MPRV	320	160	240

H = Horses

- = Negative

* = Reciprocal of serum dilution

WPV = Week post vaccination

MPRV = Month post re-vaccination