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SEROLOGICAL RESPONSES OF HORSES IMMUNIZED WITH LIVE ATTENUATED AFRICAN HORSE SICKNESS VACCINE (*)

By :

H. MIRCHAMSY and H. TASLIMI

INTRODUCTION

During the period 1959—1962, because of the widespread outbreaks of African Horse Sickness (A.H.S.), a polyvalent live, mouse-adapted vaccine was administered, on a mass scale, to Iranian and other Middle Eastern equines (Rafyi, 1961). The vaccine was prepared by Mirchamsy and Taslimi (1964a, b) and since then cell culture vaccine has been used in some African states, especially in Tunisia, Morocco and Algeria as well as in Spain, where outbreaks of A.H.S. (type 9) were reported. The cell culture live vaccine used in all these countries is produced by the A.H.S. unit of the Razi Institute (Hazrati and Ozawa, 1965).

Our early work, based on experiments with a limited number of horses, showed that polyvalent live vaccines produced with 8 mouse-adapted serological types of A.H.S. virus adapted to monkey kidney stable cell lines (M.S.) or to baby hamster kidney cell lines (B.H.K. 21), or a monovalent live vaccine produced in the same cell lines with new virus type 9, will induce a significant antibody rise for all types of viruses except for type 4 (strain V.R.Y.) which is somewhat poorer antigenically than the other types.

The present report summarizes the serological study of sera obtained from 40 horses immunized 2 years earlier with a dose of polyvalent mouse-adapted vaccine. They were revaccinated with a dose of polyvalent cell culture live vaccine. Blood samples were collected just before revaccination, 8 weeks afterwards and 2 years later. The type specific antibodies were investigated with sera collected from these blood samples. In horses, an interference phenomenon associated with types 7 and 9 was studied.

MATERIALS AND METHODS

Cell culture vaccine. The polyvalent, trivalent and bivalent live vaccines used in this study were prepared from the same virus strains previously described, i.e. type 1 (A.501), type 2 (O.D.), type 3 (L.), type 4 (V.R.Y.), type 5 (V.H.), type 6 (114), type 7 (Karen) and type 9 (S.2—Shiraz). These mouse-adapted types have been successfully adapted to M.S. cells. Passages 5 to 10 were used for vaccine preparation. Each batch contained 5×10^5 LD_{50}/ml. for adult Swiss mice.

Experimental horses. Forty healthy horses aged 7 to 10 years, immunized two years earlier with 1 dose of polyvalent mouse-adapted vaccine were used. Foals were also bought from Khorassan in North-East Iran, where no previous outbreak of the disease had been recorded.

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Serum-neutralization test. This test was carried out with sera inactivated for 30 minutes at 56°C. Twofold dilutions of each serum were made from 1:4 to 1:1,024 in phosphate buffer saline (P.B.S.) pH 7.2. The test virus contained 100 tissue culture infective dose ($TCID_{50}$). The details of the test have been described (Mirchamsy and Taslimi, 1964b).

RESULTS

Two Years after Immunization with Mouse-adapted Vaccine

The existing antibody level two years after immunization with mouse-adapted vaccine and the response to the booster dose of cell culture vaccine are illustrated in Tables 1 to 8 and Fig. 1. The data show that two years after vaccination with mouse-adapted vaccine neutralizing antibodies still existed in varying amounts. The percentage of horses with titres of 1:4 or above to types 1, 2, 3, 5, 6 and 9, were, respectively, 90, 95, 100, 95, 90 and 85 per cent. In the case of type 4 (strain V.R.Y.), already known to be a poor antigen (Mirchamsy and Taslimi, 1964b) 90 per cent. of the horses showed an antibody titre below 1:4. In the case of type 7, 70 per cent. of horses showed a titre of 1:4 or over.

TABLE 1

VIRUS TYPE 1

Antibody response of 40 horses immunized with a single dose of polyvalent tissue culture vaccine, two years after immunization with polyvalent mouse brain vaccine

Preimmunization	*Distribution of antibody titres*							*Total number immunized*	*% with 4 fold or greater response*
	Post immunization								
	1:32	*1:64*	*1:128*	*1:256*	*1:512*	*1:1024*	*>1:1024*		
<1:4			2			2		4	100
1:4		2	2	4	2	6	2	18	100
1:8		6	4	4				14	100
1:16						2		2	100
1:32						2		2	100
1:64									

TABLE 2

VIRUS TYPE 2

Antibody response of 40 horses immunized with a single dose of polyvalent tissue culture vaccine, two years after immunization with polyvalent mouse brain vaccine

Preimmunization	*Distribution of antibody titres*									*Total number immunized*	*% with 4 fold or greater*
	Post immunization										
	1:8	*1:16*	*1:32*	*1:64*	*1:128*	*1:256*	*1:512*	*1:1024*	*>1:1024*		
<1:4		2								2	100
1:4					2	2	2	2		8	100
1:8				2	4	6	4			16	100
1:16							4	4		8	100
1:32					2			2		4	100
1:64							2			2	100

TABLE 3

VIRUS TYPE 3

Antibody response of 40 horses immunized with a single dose of polyvalent tissue culture vaccine, two years after immunization with polyvalent mouse brain vaccine

Preimmunization	*Distribution of antibody titres* — *Post immunization*								*Total number immunized*	*% with 4 fold or greater response*
	1:8	*1:16*	*1:32*	*1:64*	*1:128*	*1:256*	*1:512*	*1:1024 >1:1024*		
<1:4										
1:4		2	6		2	2			12	100
1:8					6				6	100
1:16					4			2	6	100
1:32				2	6	4	4		16	87
1:64										

TABLE 4

VIRUS TYPE 4

Antibody response of 40 horses immunized with a single dose of polyvalent tissue culture vaccine, two years after immunization with polyvalent mouse brain vaccine

Preimmunization	*Distribution of antibody titres* — *Post immunization*							*Total number immunized*	*% with 4 fold or greater response*
	<1:4	*1:4*	*1:8*	*1:16*	*1:32*	*1:64*	*1:128*		
<1:4	16	6	10	4				36	55·5
1:4		2				2		4	50
1:8									

TABLE 5

VIRUS TYPE 5

Antibody response of 40 horses immunized with a single dose of polyvalent tissue culture vaccine, two years after immunization with polyvalent mouse brain vaccine

Preimmunization	*Distribution of antibody titres* — *Post immunization*								*Total number immunized*	*% with 4 fold or greater response*
	1:8	*1:16*	*1:32*	*1:64*	*1:128*	*1:512*	*1:512*	*1:1024 >1:1024*		
<1:4					2				2	100
1:4				2	6	2			10	100
1:8		2		2	4	4	6	2	20	90
1:16						2	2		4	100
1:32						2	2		4	100
1:64										

TABLE 6

VIRUS TYPE 6

Antibody response of 40 horses immunized with a single dose of polyvalent tissue culture vaccine, two years after immunization with polyvalent mouse brain vaccine

Pre-immunization	*Distribution of antibody titres — Post immunization*											*Total number immunized*	*% with 4 fold or greater response*
	<1:4	*1:4*	*1:8*	*1:16*	*1:32*	*1:64*	*1:128*	*1:256*	*1:512*	*1:1024*	*>1:1024*		
<1:4		2				2						4	100
1:4					2							2	100
1:8					2	2		2				6	100
1:16						2		4	4	6	2	18	100
1:32						2	6		2			10	80
1:64													
1:128													
1:256													
1:512													
1:1024													
>1:1024													

TABLE 7

VIRUS TYPE 7

Antibody response of 40 horses immunized with a single dose of polyvalent tissue culture vaccine, two years after immunization with polyvalent mouse brain vaccine

Pre-immunization	*Distribution of antibody titres — Post immunization*									*Total number immunized*	*% with 4 fold or greater response*
	<1:4	*1:4*	*1:8*	*1:16*	*1:32*	*1:64*	*1:128*	*1:256*	*1:512*	*1:1024*		
<1:4		4		4			4				12	100
1:4						2	2				4	100
1:8					2	6	6		2		16	100
1:16					2	2					4	50
1:32									2		2	100
1:64									2		2	100
1:128												

TABLE 8

VIRUS TYPE 9

Antibody response of 40 horses immunized with a single dose of polyvalent tissue culture vaccine, two years after immunization with polyvalent mouse brain vaccine

Pre-immunization	*Distribution of antibody titres — Post immunization*										*Total number immunized*	*% with 4 fold or greater response*
	1:4	*1:8*	*1:16*	*1:32*	*1:64*	*1:128*	*1:256*	*1:512*	*1:1024*	*>1:1024*		
<1:4		2		4							6	100
1:4						4		2			6	100
1:8						14	2				16	100
1:16							2				2	100
1:32					2			4			6	66
1:64								2	2		4	100
1:128												

Two Months after Boosting with Polyvalent Cell Culture Vaccine

Blood samples collected eight weeks after administration of cell culture vaccine showed a marked rise of antibodies of all types except type 4. Considering the preexisting low antibody titres, the response to the booster injection was high (Fig. 1, Tables 1 to 8). The greatest antigenicity was, however, observed for types 1, 2, 3, 5, 6 and 9.

TABLE 9

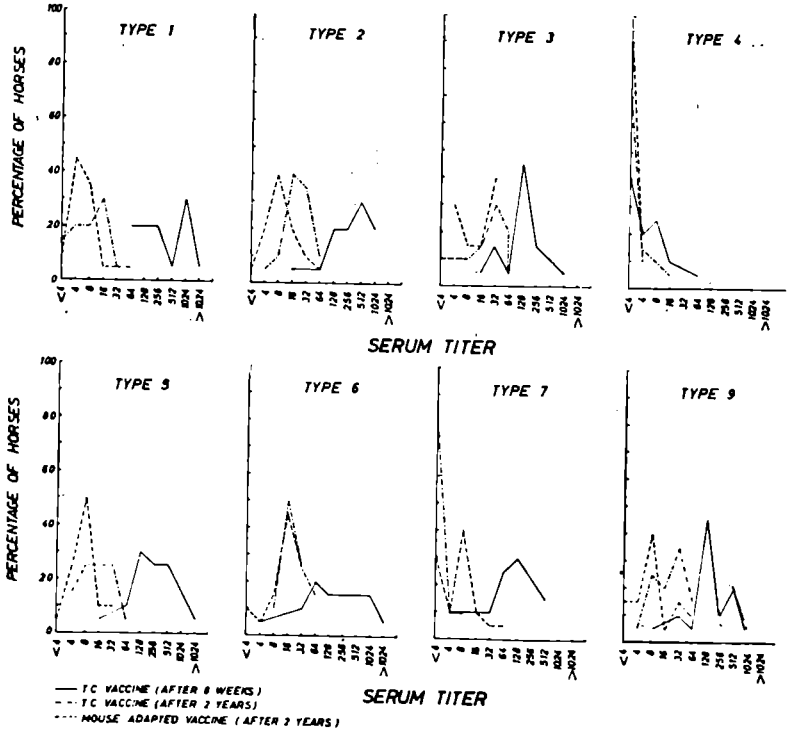
ANTIBODY TITRE OF 40 HORSES

Two years after revaccination with polyvalent cell culture African horse sickness vaccine

Antibody titre	*Type antisera-percent of horses*							
	1	*2*	*3*	*4*	*5*	*6*	*7*	*9*
<4	15	—	10	70	—	—	80	—
4*	20	5	10	15	15	—	10	5
8	20	10	10	10	25	10	10	25
16	30	40	15	5	25	50	—	20
32	5	35	30	—	25	25	—	35
64	5	10	20	—	5	15	—	15
>64	5	—	5	—	5	—	—	—

* Reciprocal of the serum dilution neutralizing 100 T.C.I.D. 50.

Fig. 1.



Two Years after Injection of Cell Culture Vaccine

The rate of decline of antibody titres, two years after inoculation of polyvalent cell culture vaccine is shown in Fig. 1 and Table 9. They indicate that the antibodies remaining after two years are somewhat above the titre found two years after immunization with mouse-adapted vaccine. There is, however, a marked breakdown in titre for type 7. This failure cannot be attributed to the poor antigenic quality of the vaccine or to the interference phenomenon since at eight weeks after revaccination with cell culture vaccine 36 out of 40 horses showed a titre of 1:16 to 1:512 for type 7.

In order to study further the immunogenicity of type 7 and because of the predominance of type 9 in outbreaks in recent years, it was decided to immunize groups of foals with cell culture bivalent or trivalent live vaccines containing type 9 and one or two other types. This procedure would also detect any possible interference between different types of A.H.S. virus. All foals were bled prior to immunization and again eight weeks after injection of bivalent or trivalent vaccine. No antibodies were detected in any of the foals before immunization.

Interference between Types 9 and 7

The antibody titres of horses immunized with 3 trivalent and 2 bivalent cell culture vaccines are grouped in Tables 10 and 11, which show marked interference between types 9 and 7. This finding confirms the observation of Ozawa (1966) who indicated interference in cell culture between these types. In these experiments we have not observed any interference between type 9 and types 1, 2, 3, 4 and 5. Type 6 was omitted since antigenic similarity has already been shown between types 6 and 9 (Howell, 1962).

TABLE 10

SEROLOGICAL RESPONSES TO CELL CULTURE

Trivalent vaccine

Foal no.	*Trivalent vaccine against types*	*Type antisera*						
		1	*2*	*3*	*4*	*5*	*7*	*9*
1	9 + 7 + 5					512	<4	512
2						512	4	1024
3						1024	<4	512
4						512	<4	64
5	9 + 4 + 3			64	<4			32
6				32	<4			128
7				128	4			1024
8				128	<4			256
9	9 + 1 + 2	64*	32					512
10		64	128					32
11		16	512					64
12		512	32					128

* Reciprocal of the serum dilution neutralizing 100 T.C.I.D. 50.

TABLE 11

SEROLOGICAL RESPONSES TO CELL CULTURE

Bivalent vaccine

Foal no.	*Bivalent vaccine against types*	*Type antisera* 5	7	9
1		1024		256
2		1024		512
3	9 + 5	N.T.		512
4		64		512
5		32		32
6		4		512
7			<4	32
8			<4	256
9	9 + 7		8	1024
10			4	256
11			<4	512
12			<4	256

N.T. = Not tested

DISCUSSION

The principle of polyvalent immunity against African Horse Sickness has been accepted since 1958 when McIntosh introduced seven distinct serological types of virus into the mouse-adapted vaccine of Alexander and du Toit (1934). The introduction of polyvalent cell culture vaccine (Mirchamsy and Taslimi, 1964a, b) was another step to control the incidence of African Horse Sickness in the equine population of many Afro-Asian countries. The distribution of several million doses of cell culture vaccine without untoward incident attests to its safety. The occurrence of the disease among unvaccinated, susceptible horses while the vaccinated animals remained resistant to the infection attests to its effectiveness.

The continuous use of polyvalent live vaccine, however, may give rise to new problems, among them the breakdown in the establishment of polyvalent immunity. Howell (1963) has indicated that animals failed to develop specific antibodies to certain components of polyvalent vaccine in spite of repeated immunizations. This failure may be due to the difference in the rates of multiplication of the various strains of virus to such an extent that cellular exclusion of one type by another may occur. This difficulty may be partly overcome by readjusting virus dosages so that immunization by all types is obtained. The selection of highly immunogenic strains is another factor which should not be overlooked. It is worth mentioning that the poor antibody responses obtained by Howell (1963) and by Mirchamsy and Taslimi (1964b) for type 4 (strain V.R.Y.) is due to the low antigenic quality of this strain, which should be replaced by a more immunogenic strain. Another point of interest is the presence of a substantial in vivo interference between type 7 (strain Karen) and type 9 (strain S.2—Shiraz). This occurred when bivalent or trivalent vaccines were injected, but was less when eight types of virus were mixed and injected in a combined vaccine.

The antibody response of cell culture vaccine in horses previously immunized with mouse adapted vaccine seems to be higher than the antibody levels due

to the mouse adapted vaccine; this relative increase in titres may be due to the antibodies present at the time of reimmunization. The breakdown in antibody titre for type 7, two years after revaccination with polyvalent cell culture vaccine, is another reason against the regular use of polyvalent vaccines.

During the most recent outbreaks of African Horse Sickness in the Middle East, North Africa and Spain the cell culture live monovalent vaccine prepared with the new type 9 proved to be safe and fully effective.

SUMMARY

In order to study the booster effects of African Horse Sickness cell culture live vaccine in horses previously immunized with polyvalent mouse-adapted African Horse Sickness vaccine, 40 horses immunized 2 years earlier with the latter were revaccinated with a single dose of polyvalent cell culture vaccine. A fourfold or higher rise in neutralizing antibody occurred in 50 to 100 per cent. of horses for all types of virus, which confirms our previous findings in foals immunized with a dose of cell culture vaccine. Response rates to the polyvalent cell culture vaccine were comparable to those of the mouse-adapted vaccine, but two years after administration of cell culture vaccine the antibody titre for type 7 was below 1:4 in 90 per cent. of horses. Interference between type 9 and type 7 was observed. These data confirm again the effectiveness of cell culture polyvalent African Horse Sickness live vaccine.

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