

## AFRICAN HORSESICKNESS VIRUS ANTIBODIES IN EGYPT (1994-1995)

(With 2 Tables)

By

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**مسح سيرولوجي لفيروس مرض النجمة في مصر عام ١٩٩٤-١٩٩٥**

تم جمع عدد ١٢٦٧ عينة من الخيول خلال عام ١٩٩٤، ١٩٩٥ والتي سبق تحصينها منذ عام ١٩٩٤ من محافظات اسوان، قنا وسوهاج ومن المحافظات التي لم تحصن منذ عام ١٩٨٤ وتشتمل على ١٨ محافظة في مصر العليا والدلتا. تم اختبار عينات السيرم باختبار الترسيب في الأجار فأعطت نتائج سلبية بالنسبة للمحافظات الغير محصنة، نسبة منخفضة بالنسبة للمحافظات التي سبق تحصينها وهي ٢.٢%. كما تم اختبار هذه العينات باختبار التثبيت المكمل فأعطت ٣.١٪، ٣.٢٪، أما اختبار الأليزا فأعطى ١.٧٪، ١.٧٪ بالنسبة للمحافظات الغير محصنة والمحافظات التي سبق تحصينها على التوالي. أجرى اختبار السيرم المتعادل على عدد من العينات الايجابية والتي سبق اختبارها فأعطت نسبة منخفضة من الأجسام المضادة لفيروس طاعون الخيل تتراوح بين ٧.٥٪ - ١٧.٥٪ للمحافظات التي لم تحصن منذ ١٩٨٤ اما في المحافظات التي أوقف بها التحصين منذ ١٩٩٤ أعطت نسبة ذودلالة تتراوح بين ١٪-٢.٦٪.

### SUMMARY

A serosurvey was done on 1267 equine serum samples collected in Egypt during 1994 and 1995 from previously vaccinated provinces, since 1994 (Aswan, Qena and Souhag) as well as from non-vaccinated provinces, since 1984 (including eighteen provinces in upper and lower Egypt). All sera were collected from non-vaccinated provinces, examined by the agar gel precipitation test (AGPT) gave negative results but previously vaccinated ones showed a low percentage of AHS specific immunoprecipitating antibodies (2.2%). Collected serum samples were subjected to complement

fixation test (CFT) and enzyme linked immunosorbent assay (ELISA) showed low percentages of African Horsesickness viral antibodies (AHSV-antibodies) (1.3, 2.3 respectively) for non-vaccinated provinces and for those from previously vaccinated ones, it was 7.1%, 17.6% respectively. Serum neutralization indices (Ni) were also estimated for some selected serum samples. Very low percentages of neutralizing antibodies were detected in sera of equines from non-vaccinated provinces (not vaccinated since 1984) which ranged from 0.75% to 1.75% and significant percentages and titers were in sera from horses in previously vaccinated provinces (Vaccination was stopped by Jan. 1994) which were from 1% to 2.60%.

*Key words: African Horsesickness-Antibodies-Egypt.*

## INTRODUCTION

African horsesickness (AHS) is an acute or subacute infectious, non contagious, arthropod-born viral disease of equidae. The disease is transmitted by blood sucking arthropods. Two groups of biological vectors, culicoides and mosquitoes are highly suspected (Dutoit, 1944 and Ozawa *et al.*, 1966). Nine serotypes of AHS virus have been identified (Mcintosh, 1958 and Howell, 1962). The disease occurs endemically in countries of southern and central Africa. Last waves of epizootics were recorded in south of Spain, Portugal and Morocco between 1987 and 1989 (Oie, 1989). The causative agent was AHS serotype 4. Also AHS epizootics were reported in Saudi Arabia in 1989 where AHS virus serotype 9 was identified (Anderson *et al.*, 1990). Later on, a minor epizootic of AHS was reported by Farid *et al.*, (1981) in Aswan in 1971, the isolates were identified as AHS virus serotype 9.

In Egypt, from 1971 and up to 1983, the veterinary authorities was following a policy of annual mass vaccination of equines in all Egyptian provinces using a polyvalent attenuated tissue culture vaccine of AHS virus, (serotype 1-9). In 1984, the vaccination regime was only confined to Aswan, Qena and Souhag provinces. At the beginning of 1994, the responsible authorities stopped the vaccination programme against AHS in order to create a zone free from AHS viral antibodies to facilitate the traveling of horses from Egypt to different countries all over the world. Thus, this work is a field survey planned to cover the enzootic area in Aswan and eighteen other provinces in upper and lower Egypt, by detecting specific antibodies of AHS using various serological test (CFT, AGPT, ELISA and SNT).

## **MATERIAL and METHODS**

### **1- Serum Samples:**

1267 serum samples were examined from groups of animals representing different ages and different provinces (previously vaccinated since, 1994) and non vaccinated provinces (since 1984).

### **2- Viruses:**

Nine reference mouse adapted neurotropic vaccinal strains of AHS virus serotypes (A501), 2 (OD), 3 (KA), 4 (Vryheid), 5 (Valhart), 6 (114) and 7 (Karen) were obtained from Onderstepoort Vet. Inst. at its 100<sup>th</sup> mouse passage. Types 8 (18/60) and 9 (S2) were received from US Dept. of Agricultural Research Service. A few additional passages of these strains were made in mice for the preparation of fresh seed virus to be used for adaptation on VERO cell cultures. The infectivity titer of the viruses calculated according to the method described by Reed and Muench (1938).

### **3- Antisera:**

Antisera against each of the nine neurotropic viruses representing the mouse adapted vaccinal strains, were prepared from hyperimmunized rabbits (from type 1 to 7) and goats (types 8, 9) according to the method described by Salama *et al.* (1976).

### **4- Serological tests:**

Enzyme linked immunosorbent assay (ELISA) was applied according to the procedure of William (1987) and Carol *et al.* (1990). A complement fixation test (CFT) was carried out according to the techniques of Salama *et al.* (1979). Agar gel precipitation (AGPT) was performed according to Salama *et al.* (1977). Serum neutralization test was done according to the microtechnique of Mellor *et al.* (1990).

## **RESULTS**

Results of testing equine serum samples for the presence of specific AHS antibodies are shown in Tables (1 and 2).

## DISCUSSION

Since 1960, the Veterinary Authorities followed a policy to vaccinate equine species in most of the provinces to protect them against natural infection with AHSV. However, later on in 1984, they changed the vaccination policy and decided to vaccinate equine population in only 3 provinces :Aswan, Qena and Souhag. Moreover, the Veterinary Authority decided to stop the vaccination program in all the provinces of Egypt since 1994. This was under taken to create a free zone which proved to be efficient since no cases or outbreaks appeared during the period from 1984 (in the non-vaccinated provinces) up till now.

However, we should determine the immune status of the equine population so that, a correct judgement on the degree of immunization and the level of antibodies could be reached. This could not be achieved unless one carries a serosurvey on random serum samples from previously vaccinated as well as non vaccinated provinces. In addition, this serosurvey may give an information on the probability of a subclinical infection due to circulating virus or reactivation of a vaccinal strain (post vaccination) or even due to other viruses sharing common antigenicity with AHS viruses (Ozawa and Hafez, 1973). In the present study a total number of 1267 equine serum samples were collected during 1994 and 1995 from the previously vaccinated provinces (Aswan, Qena and Souhag since 1994) and eighteen non vaccinated provinces (since 1984). The obtained data showed that both CFT and ELISA gave a more precise picture about the incidence of AHS antibodies since these two techniques revealed comparable results i.e the result of CF and ELISA conducted on horse serum samples collected from previously vaccinated provinces showed high percentages of positive cases ranging 4-11.4% and 8% to 30.5% for the two tests respectively. Lower percentages of positive cases were illustrated for non-vaccinated ones (2% to 6.7%) and (3.3 % to 13.3 %) respectively. When applying the AGPT on the same serum samples, precipitating bands could only be detected with Aswan (4.8%) and Qena (2.8%) while the other provinces gave negative results. Moreover, the results of neutralization test showed positive results with most serotypes of AHS virus, specially with samples from previously vaccinated horses (Aswan, Qena and Souhag since 1994). The presence of high percentages of CF, ELISA and neutralizing antibodies against AHSV in serum samples collected from previously vaccinated provinces may be due to the strict annual vaccination before 1994. While, the low percentages of CF, ELISA and neutralizing antibodies in sera of horses collected from non-

vaccinated ones be due to the movement of vaccinated horses or infected arthropods from southern to northern provinces which agree with Parker (1974) who detected complement fixation and neutralizing antibodies in sera from vaccinated horses 11 years post-vaccination in Cyprus. The absence of CF, PP, ELISA and neutralizing antibodies in 90% of non vaccinated provinces and low titers of AHS antibodies in the rest of provinces when compared with the titers of infected or vaccinated animals gave us an idea about that AHS viruses did not persist in Egypt.

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Table ( 1 ) : Results of testing serum samples by AGPT, CFT and ELISA for presence of antibodies against AHS virus.

Immune status of animals	Governorate	Results of serological tests for each province					
		AGPT		CFT		ELISA	
		* x	%	x/y	%	x/y	%
Previously vaccinated (Since 1994)	Aswan	5/105	9.8 %	12/105	11.4 %	32/105	30.5 %
	Qena	3/107	2.8 %	7/107	6.6 %	15/107	14.0 %
	Souhag	0/100	0.0 %	4/100	4.0 %	8/100	8.0 %
Cumulative		8/312	2.2 %	23/312	7.1 %	45/312	17.6 %
Not vaccinated since 1984 (Upper and lower Egypt)	Assiut	-	-	4/60	6.7 %	8/60	13.3 %
	El-Minya	-	-	0/55	0.0 %	0/55	0.0 %
	Beni-Suef	-	-	0/60	0.0 %	0/60	0.0 %
	El-Fayum	-	-	0/60	0.0 %	0/60	0.0 %
	Giza	-	-	2/60	3.3 %	2/60	3.3 %
	Kalioubieh	-	-	0/55	0.0 %	0/55	0.0 %
	Sharkieh	-	-	0/55	0.0 %	0/55	0.0 %
	Monofia	-	-	1/50	2.0 %	2/50	4.0 %
	Dakahlieh	-	-	0/50	0.0 %	0/50	0.0 %
	Beheria	-	-	0/50	0.0 %	0/50	0.0 %
	Charbieh	-	-	0/50	0.0 %	0/50	0.0 %
	Matrouh	-	-	3/50	6.0 %	4/50	8.0 %
	North Sinia	-	-	0/50	0.0 %	0/50	0.0 %
	Alexandria	-	-	0/50	0.0 %	0/50	0.0 %
Port Said	-	-	2/50	3.3 %	5/50	10.0 %	
Suez	-	-	0/50	0.0 %	0/50	0.0 %	
Cairo	-	-	0/50	0.0 %	0/50	0.0 %	
Ismailia	-	-	0/50	0.0 %	0/50	0.0 %	
Cumulative		0/955	0.0 %	12/955	1.3 %	21/955	2.3 %

\* : Number of tested serum samples.

\*\* : Number of positive serum samples.

Table ( 2 ) : ABS virus neutralizing antibodies in sera from previously vaccinated and non vaccinated horses.

Immune status of animals	Governorate	Mean N-index (NI <sub>50</sub> )									
		Serotypes									
		1	2	3	4	5	6	7	8	9	
Previously vaccinated provinces, since 1994	Aswan ** (10)	*	1.50	1.00	0.75	1.75	2.25	1.50	2.25	1.75	2.50
	Qena ** (10)	*	2.00	1.75	2.00	2.60	2.25	2.25	2.00	2.50	2.50
	Souhag ** (5)	*	1.50	1.25	1.25	1.75	1.00	1.60	1.50	1.00	1.25
Non vaccinated provinces, since 1984	Assiut ** (5)	-	-	-	-	-	-	-	-	-	-
	Giza ** (5)	-	-	-	-	-	-	-	-	-	-
	Monifia ** (5)	-	-	-	-	-	-	-	-	-	-
	Port Said ** (5)	0.75	0.50	1.00	1.25	1.00	1.75	1.00	0.75	1.75	
	North Sinia ** (5)	-	-	-	-	-	-	-	-	-	-

\* : Log NI

\*\* : Number of tested serum samples.

