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Studies on arboviruses in Egypt

II. Contribution of arboviruses to the aetiology of undiagnosed fever among children

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SUMMARY

Acute blood samples from 120 children, attending the fever hospital in Alexandria and complaining of fever, were collected and examined for haemagglutination-inhibiting (HAI) and complement-fixing (CF) antibodies against the following arbovirus antigens; Sindbis, West Nile (WN), yellow fever, dengue 1, sandfly fever, Quaranfil, Chenuda and Nyamanini. Positive reactions in the acute sera were only detected against Sindbis (4·3%) and WN (4·3%) antigens. The convalescent sera obtained from 48 of these children showed a pronounced HAI titre against WN antigen in 14·6% of them. The same sera showed a lower titre against yellow fever antigen (Asibi strain) which is due to cross-reaction between the two viruses. None of the acute or the convalescent sera showed CF antibodies against Quaranfil, Chenuda or Nyamanini antigens. The convalescent sera were not tested against dengue type 1 antigen. It is suggested that of the known arboviruses in Egypt, WN is the most important from the public health point of view.

INTRODUCTION

In continuation of our study on arboviruses in Egypt (Mayer *et al.* 1967; Mohammed, Sekeyová, Grešiková & El-Dawala, 1968), it was found necessary to evaluate the contribution of these viruses to the aetiology of undiagnosed fever among children. Usually, a large number of children complaining of fever, daily attend the fever hospital in Alexandria. The results of previous serological examination of the human adult population of Alexandria showed the presence of haemagglutination-inhibiting antibodies against sandfly (45%) and West Nile (16%) antigens (Mohammed *et al.* 1968).

In the present investigation, acute and convalescent blood samples were tested with the following arboviruses: Sindbis, West Nile, yellow fever, dengue type 1, Sicilian sandfly, Quaranfil, Chenuda and Nyamanini antigens.

MATERIALS AND METHODS

Human blood sera

Acute-stage blood samples were collected during the months of June to October 1968, from 120 children (60 males, 60 females; 3–13 years of age) attending the fever hospital in Alexandria. Convalescent sera from some of the same children (24 males, 24 females) were collected one and a half months later. Acute sera were stored frozen at $-20°$ C. until the collection of the convalescent sera; both were then tested immediately.

Serological tests

Human sera were tested for the presence of haemagglutination-inhibiting and complement-fixing antibodies.

Complement fixation (CF) test

The standard method using plastic plates was followed. The plates with the sera, antigens and two units of complement were allowed to stand overnight at 4° C. before the addition of the haemolytic system. All sera were absorbed by kaolin before the CF test, since they were found to be anticomplementary.

Haemagglutination inhibition (HAI) test

Antigens for haemagglutination (HA) and haemagglutination inhibition (HAI) tests were prepared by the sucrose acetone extraction method from the brains of infected unweaned mice according to the techniques described by Clarke & Casals (1958). Sera were extracted by acetone and absorbed with goose erythrocytes prior to testing. The HA and HAI tests were carried out by the method of Clarke & Casals (1958) as modified for use in plates.

Virus strains used

Sindbis virus. This is the passage number 2 in unweaned mice at the Rockefeller Foundation Virus Laboratory (RFVL). It has undergone six passages in unweaned mice at the Regional Reference Laboratory (RRL) in Bratislava.

West Nile virus. Egypt 101. Passage number 7 in unweaned mice at the RFVL. It has undergone 12 passages in baby mice at the RRL in Bratislava.

Dengue 1 virus. Originally obtained from the RFVL, passage number 11 in newborn mice, it has undergone a further five passages at the RRL in Bratislava.

Yellow fever virus. Asibi strain. It has undergone four passages in baby mice at the RRL in Bratislava.

Sandfly fever virus. Sicilian phlebotomus fever, Sabin strain, 37 passages at the RFVL, further two passages at the Instituto Superiore di Sanita, Rome, and three passages at the RRL in Bratislava.

Nyamanini virus. (Eg Ar 1304, P-25). It has undergone three passages in baby mice at the RRL in Bratislava.

Quaranfil virus. (Eg Ar 1113, P-6). It has undergone three passages in baby mice at the RRL in Bratislava.

Chenuda virus. (Eg. Ar 1152, P-18). It has undergone two passages in baby mice at the RRL in Bratislava.

The last three viruses were kindly sent by Dr Karabatsos, Yale Arbovirus Research Unit.

RESULTS

In the acute sera from children, haemagglutination-inhibiting antibodies were detected against Sindbis antigen in 4 % and against West Nile antigen in 4 %. No antibodies were found against yellow fever, dengue type 1 or sandfly antigens. Similarly, no complement-fixing antibodies could be observed with Quaranfil, Chenuda or Nyamanini antigens. The results of the HAI and CF tests with acute sera are given in Table 1.

Table 1. *Haemagglutination-inhibiting and complement-fixing antibodies in acute sera of children, Alexandria,* 1968

Antigen	Titre	Number of sera	Positive (%)
Sindbis	< 20	115	—
	20	1	4
	40	2	
	80	2	
West Nile	< 20	115	—
	20	1	4
	40	1	
	80	3	
Yellow fever	< 20	120	0
Sandfly fever	< 20	120	0
Dengue type 1	< 20	120	0
Quaranfil*	< 20	120	0
Chenuda*	< 20	120	0
Nyamanini*	< 20	120	0

* Tested by complement fixation.

Table 2. *Titres of haemagglutination-inhibiting antibodies in acute and convalescent sera which were positive with West Nile antigen, Alexandria,* 1968

Antibody titres in		Age of child years
Acute serum	Convalescent serum	
< 20	640	10
< 20	640	4
< 20	640	5
< 20	640	13
< 20	640	11
< 20	1280	6
< 20	1280	9

In the convalescent sera, on the other hand, seven out of 48 children tested (15 %) showed HAI antibodies against West Nile antigen (Table 2). These same sera showed lower titres against yellow fever antigen, but they and all the other

convalescent sera were negative by the HAI test against Sindbis and sandfly antigens, and by the CF test against Quaranfil, Chenuda and Nyamanini antigens (Table 3).

Table 3. *Haemagglutination-inhibiting and complement-fixing antibodies in convalescent sera of children, Alexandria,* 1968

Antigen	Antibody titre	Number of sera	Positive (%)
West Nile	< 20	41	—
	20	0	—
	40	0	—
	80	0	—
	160	0	—
	320	0	—
	640	5	15
	1280	2	
Yellow fever	< 20	41	—
	20	0	—
	40	4	15
	80	1	
	160	2	
Sindbis	< 20	48	0
Sandfly fever	< 20	48	0
Quaranfil*	< 20	48	0
Chenuda*	< 20	48	0
Nyamanini*	< 20	48	0

* Tested by complement fixation.

DISCUSSION

In the previous serological survey among the urban population of Alexandria, HAI antibodies were found against sandfly fever in 45% and West Nile virus in 16%. The results of the present investigation showed that out of arboviruses known to be present in Egypt, West Nile virus is probably the most important from the public health point of view. The positive convalescent sera showed high titres of HAI antibodies to WN antigen; in five of them a value of 640 and in the other two a value of 1280 (Table 2). It was also noted that although the sera were collected during the period June to October 1968, all positive sera were obtained during August. This is in agreement with the findings of Taylor, Work, Hurlbut & Farag Risk (1956), who described West Nile virus isolations from human blood with the highest percentage during July and August. It should be pointed out that the children with HAI antibodies in their acute sera against WN and Sindbis viruses did not give us convalescent sera.

Cross-reactions with the B group of arboviruses were observed with yellow fever antigen (Asibi strain) in convalescent sera which contained HAI antibodies against West Nile virus. The titre of antibodies against West Nile virus was in all samples higher than with yellow fever antigen. None of the acute or the convalescent sera contained complement-fixing antibodies to Quaranfil, Chenuda or Nyamanini antigens. This finding may indicate that in the sera investigated,

there is a very low contact of humans with these viruses. With the exception of two Quaranfil virus strains (H-2351, H-8762) all of these viruses were isolated from ticks, nestling egrets or pigeon squab (Taylor *et al.* 1966). It is of interest to mention that both Quaranfil and Nyamanini viruses have been also isolated from one and the same egret rookery in South Africa.

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