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Behaviour of large and small plaque variants of Sindbis and Western Equine Encephalitis viruses in *Aedes aegypti*

BY

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Summary — Large plaque variants of Sindbis and WEE viruses are more infectious for *A. aegypti* mosquitoes than the corresponding small plaque variants.

These results together with those formerly obtained with Middelburg virus sustain the idea that the large plaque variant of group A arboviruses is generally the one occurring in nature.

Introduction

In a previous paper were presented the results of the infection of *A. aegypti* after feeding large (l) and small (s) plaque variants of Middelburg virus.

We have since extended these observations on two other arboviruses producing l and s plaque types under agar in chick embryo tissue culture (CETC).

Materials and methods

Tissue culture techniques, virus strains used and the technique for feeding viruses to mosquitoes have been described earlier (Pattyn and De Vleesschauwer : 1966, 1967, 1968).

Results

Table 1 shows the results obtained with Sindbis virus. It appears that the large plaque virus multiplies readily in *A. aegypti* : the 50 percent threshold of infection is 3.10^7 PFU/ml of virus in the infecting blood meal, whereas this value is higher than 10^9 PFU/ml for the small plaque variant.

To exclude the possibility that the s type virus used consisted of a mixture of a few virus particles multiplying readily in *A. aegypti*,

TABLE 1

Sindbis virus in *A. aegypti*

Virus Titer Blood meal PFU/ml	Number of virus positive mosquitoes after 4 and 5 weeks							
	si-1	%	si-s	%	si-wild	%	si-s mosq (*)	%
$1.2\ 10^9$			8/30 (**)	26				
$4.6\ 10^8$			2/27	7				
$2.7\ 10^8$							2/46	4,3
$1.4\ 10^8$					1/31	3		
$5.2\ 10^7$			0/29					
$3\ 10^7$	21/42	50						
$1.4\ 10^7$	2/18	11						
$1.1\ 10^7$					2/35	5		
$4.5\ 10^6$			0/6					
$1.4\ 10^6$					1/31	3		

(*) = si-s virus recovered from a mosquito and refed to mosquitoes after one newborn mouse passage.
(**) = nominator : mosquitoes positive for virus denominator : total number of mosquitoes tested.

among a population not readily multiplying in the mosquitoes, we passed the harvest from a positive mosquito once in baby mice (to obtain a high amount of virus) and refed this to *A. aegypti.*

Column 4 in table 1 shows that this virus did not behave differently as compared with s virus that had not passed through mosquitoes.

The threshold of infection for mosquitoes of a non plaque purified strain of Sindbis virus maintained in our laboratory and with a history of an unknown number of passages in mice was also very high.

Virus titers of individual mosquitoes infected with Sindbis-l virus were of the order of 10^4 - 10^5 PFU per mosquito as compared with 10^2 - 10^3 PFU per mosquito in the case of Sindbis-s virus.

Table 2 shows the results obtained with two plaque variants of WEE virus. The large plaque virus multiplied to a significant extent in our *A. aegypti* mosquitoes producing titers of 10^5 PFU per individual insect.

TABLE 2

WEE virus in *Aedes aegypti*

Virus titer Blood meal PFU/ml	Number virus positive mosquitoes after 4-5 weeks		
	WEE-l	%	WEE-s
6 10^8/ml	41/44 (*)	93	
1.9 10^8			0/19
1.7 10^8	33/43	76	
1.8 10^7			2/29
10^7	1/29		
3.5 10^6	0/15		
1.3 10^6			0/20
4.9 10^5	0/26		

(*) = nominator : mosquitoes positive for virus denominator : total number of mosquitoes tested.

In contrast only 2 out of 48 mosquitoes infected with the higher amounts of WEE-s virus were positive after 4 and 5 weeks. These mosquitoes titered only 3.2 10^2 and 1.2 10^3 respectively.

Moreover in once instance of mosquitoes fed WEE-l virus (titer of the infectious meal 1.7 10^8 PFU/ml) was virus recovered which produced intermediate sized plaques : 3.5 mm diameter after three days incubation, as compared with the 7-8 mm diameter of the originally fed WEE-l virus.

This particular virusstrain awaits further study.

Discussion

The observation on the behaviour of l and s plaque variants of group A arboviruses has now been extended to three viruses : Middelburg (Pattyn and De Vleesschauwer, 1968), Sindbis and WEE.

In each case the l plaque variant is significantly more infectious for *A. aegypti* than the corresponding s plaque virus. These observations sustain the idea that the large plaque variant is the naturally occurring virus.

We are aware of only two results on this subject in the literature : Marshall and his colleagues (1962) found that WEE virus naturally occurred as large plaque virus, while Ushijima and colleagues (1962) mention that WEE virus isolated from mosquitoes measured 2 - 5 mm. (intermediate between l and s plaques).

The 50 percent thresholds of infection for the mosquitoes are rather high : about 10^7 PFU per ml in each favorable case.

Various factors intervene in determining this threshold level : passage history of the virus, the composition of the infectious meal (Chamberlain and Sudia, 1961), the combination virus-mosquito species which is not necessarily optimal and the route of infection of the mosquito : *per os* or through injection.

The influence of the passage history of the virus used is illustrated by our laboratory strain of Sindbis virus which was much less infectious than the Sindbis l plaque purified virus.

A. aegypti is not a common natural vector, for neither of the two viruses studied, most Sindbis virusstrains have been isolated from *Culex* species and the most common source of WEE virus are *Culex tarsalis* and *Culex melanura*. *Aedes aegypti* can however be readily infected with WEE virus. Chamberlain and his colleagues (1954) using a strain of WEE virus that had only two mouse brain passages, found that 100 percent of *A. aegypti* were infected after feeding on chicks with a blood titer of 10^7 - 10^8 mouse intracerebral LD50 (= per 0.03 ml) a figure which compares favorably with our results.

Samenvatting — **Gedrag van grote en kleine plaque varianten van Sindbis en WEE virussen in *A. aegypti*.**

Grote plaque varianten van Sindbis en WEE virus zijn veel besmettelijker voor *Aedes aegypti* dan de kleine plaque varianten van dezelfde virusstammen.

Deze resultaten zijn analoog met wat voorheen voor Middelburg virus varianten werd waargenomen en steunen de gedachte dat de grote plaque varianten in de regel de natuurlijk voorkomende vorm van groep A arbovirussen zijn.

Résumé — **Comportement des variants à petites et grandes plaques des virus Sindbis et WEE chez *A. aegypti*.**

Les variantes à grandes plages des virus Sindbis et WEE sont beaucoup plus infectieux pour *Aedes aegypti* que les variants à petites plages des mêmes virus.

Ces résultats sont analogues à ceux obtenus précédemment avec les variants du virus Middelburg et soutiennent l'idée qu'en règle générale les virus à grandes plages sont la forme naturelle des arbovirus du groupe A.

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