RECENT STUDIES OF HAEMORRHAGIC FEVERS IN SINGAPORE*

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Haemorrhagic fever in the Philippines was described by Hammon et al, 1960 a, and a similar disease was reported in Thailand by Nelson et al, 1960. The virus studies in these two diseases were reported by Hammon et al, 1960 b, and summarised at the Tenth Pacific Science Congress. This report is of a related disease which first appeared in Singapore in 1960. The clinical features have been described by Chew et al, 1961 and also by Lim, Hanam & Chan, 1961 and are only summarised here with results of more recent studies.

The Singapore outbreak began in June, 1960 and is best described as a febrile illness resembling dengue but with haemorrhagic manifestations.

The patients mostly present with a febrile illness with headache, backache and remote pains, some with lymphadenopathy. During the course of the fever about half the patients developed morbilliform rashes characteristic of dengue fever and the disease would have been regarded as dengue fever but for the appearance of petechial haemorrhages in a good many patients. In a series of 79 patients studied by Lim et al, 1961, 16 had petechial haemorrhages but there was no frank bleeding. All had thrombocytopenia with thrombocyte counts of less than 200,000 per cu mm by the direct method. 63% had thrombocyte counts of less than 100.000 per cu mm. The patients were mainly young adults and there were no deaths. The clinical features are summarised in Fig. 1.

The disease had never been seen before in Singapore and was named Singapore Haemorrhagic Fever to distinguish it from dengue fever which is endemic in Singapore and in Malaya. This has been shown by serological studies, Hale et al, 1956, although dengue virus had not been isolated before in Singapore. An epidemic of dengue fever was reported in Singapore in 1905 when no virus studies were available. In the Federation of Malaya there was an outbreak of dengue fever in 1956 when Smith isolated dengue type 1 virus.

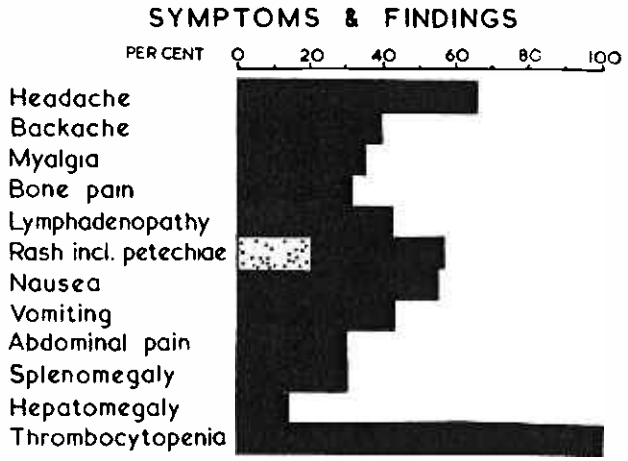

Fig. 1. Clinical features in 79 cases of Singapore Haemorrhagic Fever.

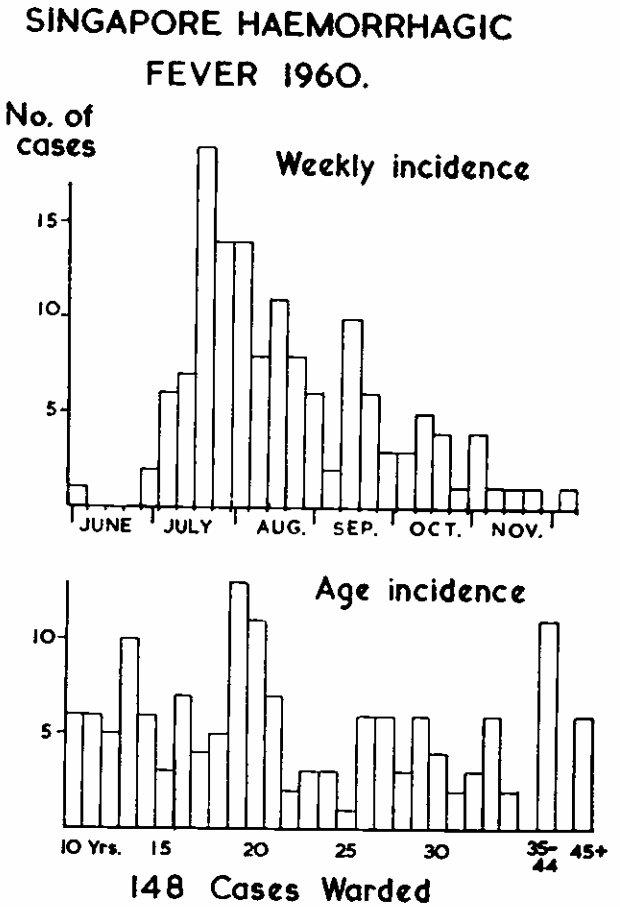

Fig. 2. Weekly incidence by date of onset and age incidence.

* Presented at the 10th Pacific Science Congress, Hawaii, 1960 in the Division of Public Health and Medical Sciences.

59 paired sera from the series of 79 patients studied by Lim et al were examined for complement fixing antibodies for Japanese B encephalitis virus (JEV) and dengue type 1 virus. The complement fixation tests (CFT) were done by the drop method on plastic trays using 2 units of antigen prepared by Casals freeze and thaw technique (Casals, 1947). 24 patients were positive for dengue type 1 antigen only with CFT titres ranging from 1/32 to more than1/256 and 13 of the paired serum specimens showed more than 4-fold antibody rise. 27 other patients were positive for both JEV and dengue type 1 virus antigens. 8 patients were negative for both antigens. The serological reactions suggested strongly that dengue type viruses were responsible for the disease. The positive reactions for JEV was not unexpected in view of the well known crossing between viruses in Casal's group B to which both JEV and dengue viruses belong.

By the end of 1960 148 cases including the 79 cases mentioned had been studied in Singapore General Hospital. The weekly incidence and the age incidence are shown in Fig. 2. There were 10 cases below the age of 10 years which have not been included in this study or in the figures. All cases studied were hospital admissions and it is not known how many cases there were who did not seek hospital treatment. The age incidence is in marked contrast with that of the Philippines and Thailand diseases in which frank bleeding was a prominent feature and where mostly young children were affected with case mortality of about 10%. However, Nelson reported splenomegaly, hepatomegaly and abdominal pain in Thailand. It is clear that Singapore Haemorrhagic Fever is quite a different disease and it should be pointed out also that it is different from Korean Haemorrhagic Fever in which a renal syndrome was involved.

About 80% of the cases occurred in the urban and suburban areas and most of them in the built up areas. Fig. 3 shows the distribution of haemorrhagic fever cases compared with that of 65 Japanese B encephalitis cases that occurred between 1956 and 1960 and had been diagnosed by serological studies or virus isolation from autopsy material.

The difference in distribution of the two diseases is very striking. Only 6, in fact, of Japanese B encephalitis cases occurred in the built up areas although about 30% of the 65 cases shown were in the City or urban and suburban areas. The evidence suggests strongly that if a mosquito vector was involved in the haemorrhagic fever

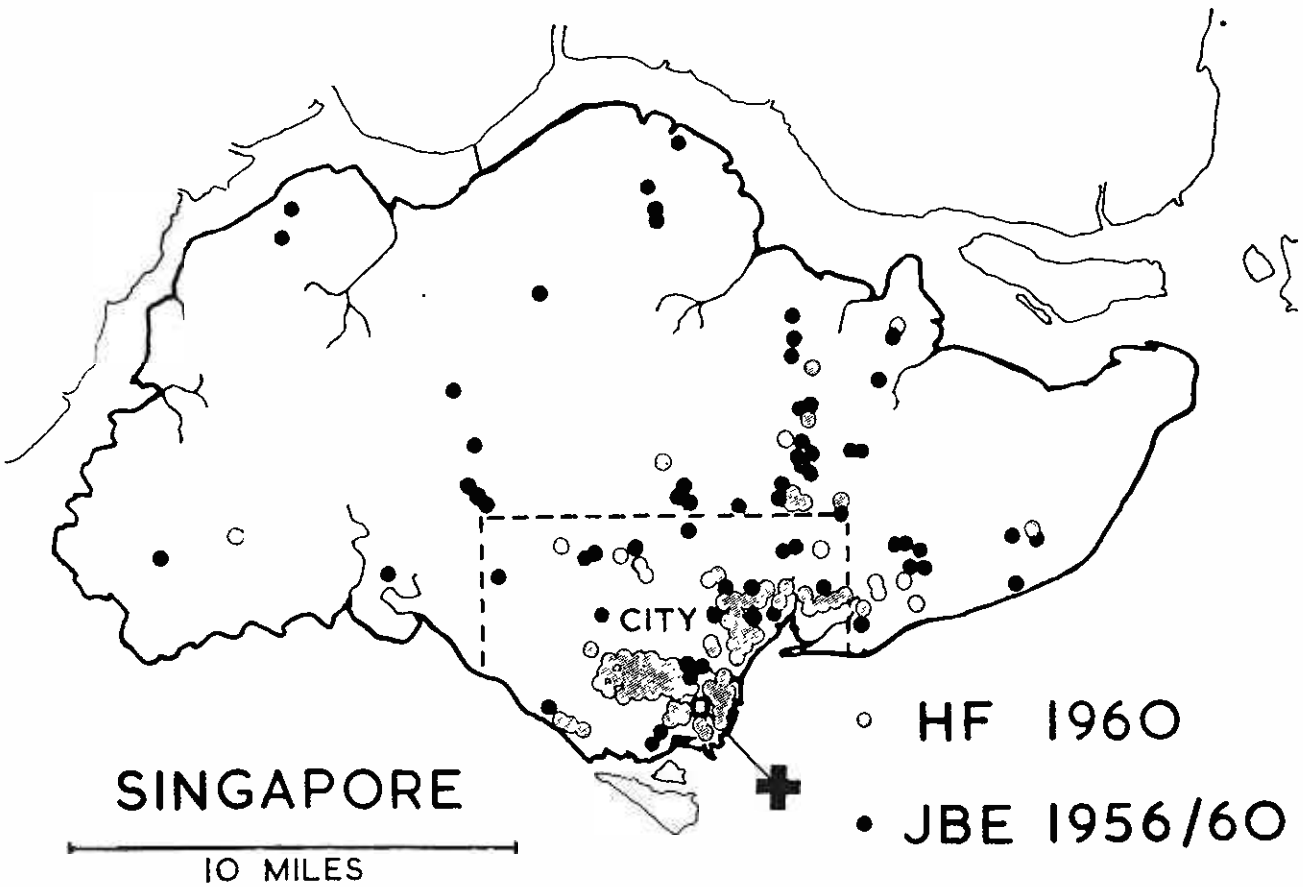

Fig. 3. Distribution of cases of 148 cases of Singapore Haemorrhagic Fever and 65 cases Japanese B. encephalitis.

outbreak, it must have been an urban mosquito. Entomological studies initiated in October 1960 showed that Aedes aegypti were common in the urban area being found in more than 50% of the houses visited. Fig. 4 lists the common species that were found. Attention is drawn to the fact that although Culex fatigans and Aedes albopictus were commonly found in both urban and rural areas, the distribution of the cases suggests that Aedes aegypti was the only species that could be incriminated as the vector of Singapore Haemorrhagic Fever. It may be noted also that the occurrence of Culex tritaeniorhynchus, well known to be a vector for Japanese B encephalitis is consistent with the distribution of this disease.

Virus isolations from mosquito pools were attempted by inoculation of infant mice, 15 pools of Aedes aegypti having been processed so far. Four of these pools demonstrate possible presence of dengue related viruses in that over 50% of surviving mice were immune to intracerebral challenge by adult adapted dengue type 1 virus. Blind passage is being continued.

Initial attempts to isolate virus from human material included inoculation of tissue culture with throat washings and faecal extracts. These methods were discontinued when the serological results were known and attempts to isolate virus from serum specimens were intensified. Blood or serum taken within the first five days of illness was inoculated intracerebrally and subcutaneously in one to two-day old infant mice. Two viruses S. 601/60 and S. 843/60 were isolated and adapted to adult mice within 7 to 10 passages. The patients from whom these viruses were isolated had CF and neutralising antibody rises for these virus isolates as well as for dengue virus types 1, 2, 3, and 4 and JEV (Fig. 5). An unexpected result was the antibody rise in patient S. 601 for chikungunya virus by CFT and neutra-

COMMON MOSQUITO SPECIES IN SINGAPORE

URBAN	Culex fatigans Aedes aegypti Aedes albopictus
SUBURBAN & RURAL	Culex fatigans Aedes albopictus Anopheles vagus Culex gelidus Culex tritaeniorhynchus Mansonia uniformis

Fig. 4 Common mosquito species found in Singapore

Fig. 5 Virus studies of two patients.

Patient Sex/Age	Virus Isolation	Serum days	Dengue I NA	Dengue I CF	Dengue II NA	Dengue II CF	Dengue III NA	Dengue III CF	Dengue IV NA	Dengue IV CF	JBE NA	JBE CF	S-601 NA	S-601 CF	S-843 NA	S-843 CF	Chikungunya NA	Chikungunya CF
S-601 M 25 yrs	Dengue I ?	2	< 1.1	< 4	—	< 4	—	< 4	1.0	< 4	—	4	0.7	< 4	—	< 4	0.7	8
		17	> 3.3	1024	—	> 2048	—	1024	> 3.4	512	—	1024	3.5	1024	—	512	2.7	16
		31	—	512	—	> 2048	—	1024	—	512	2.9	512	2.8	1024	—	256	—	16
		45	—	512	—	> 2048	—	1024	—	256	—	512	3.0	512	—	256	—	32
S-843 M 24 yrs	Dengue II ?	1	—	< 4	0.5	< 4	—	4	1.2	< 4	—	< 4	—	< 4	1.8	< 4	—	< 4
		43	—	512	2.7	2048	—	1024	4.8	1024	1.0	512	—	512	3.1	512	—	< 4

NA = Neutralising antibody expressed as log Neutralisation Index, determined in adult mice except for Dengue III where infant mice were used.
Prototype viruses: Dengue I, Hawaii; Dengue II, N.G.C.; Dengue III, H-87; Dengue IV, H-241; JBE, Nakayama.
CF = Reciprocal of complement fixing titre. Plate techniques were used, test mixtures being held overnight in the cold.
— = not tested.

lisation tests (NT). This was the only positive result in 17 patients tested and its significance cannot be assessed until further studies are done.

The identification of the virus isolates have been carried out through the preliminary stages. Fig. 6 shows the conventional checkerboard CFT for antigenic relationships. Although there are, as may be expected, some minor cross reactions with other viruses, S. 601 is clearly shown to be most closely related to dengue type 1 virus and S. 843 to be most closely related to dengue type 2 virus. On the other hand there are no cross reactions between the isolates. Fig. 7 shows that cross-neutralisation tests give the same antigenic relationships.

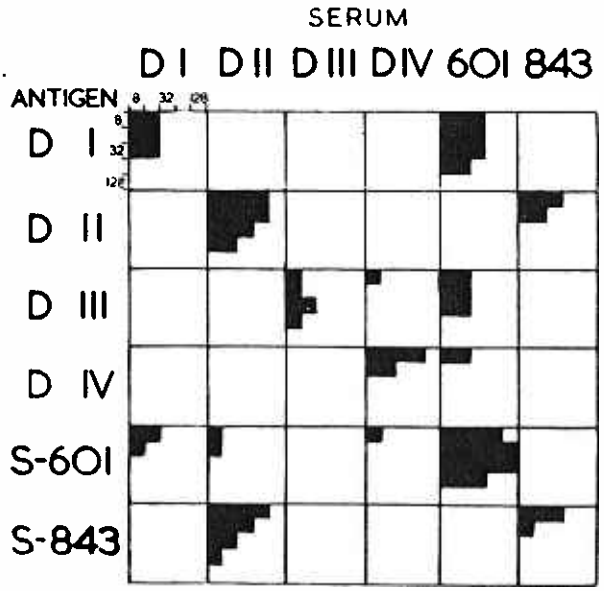

Fig. 6. Comparison of two virus isolates with dengue viruses by cross complement fixation tests.

Immune serum	Virus & Log Neutralization Index					
	D I	D II	D III	D IV	S-601	S-843
Dengue I	3·4	1·2	O	O	2·6	O
Dengue II	O	2·8	O	O	O	2·5
Dengue III	1·3	1·9	<1·8	0·9	1·8	1·2
Dengue IV	1·1	1·5	0·9	4·5	1·5	1·8
S-601	1·7	1·1	O	O	1·5	O
S-843	O	2·1	O	1·1	O	3·9

Fig. 7 Comparison of two virus isolates with dengue viruses by cross-neutralisation tests.

O = Log Neutralisation index of less than 0.5.
S.601 serum was G.P. serum, the other sera were mice sera.

DISCUSSION

A new disease appeared in Singapore in 1960 in which at least two dengue type viruses were involved and possibly chikungunya virus. The disease resembled dengue fever but had haemorrhagic manifestations hitherto not seen in sporadic cases of dengue fever. The disease was named Singapore Haemorrhagic Fever but was different from the haemorrhagic fevers of Thailand and of the Philippines. However, the diseases in all three countries were similar in that dengue viruses of more than one type were isolated in each of them. It is apparent that the dengue viruses may cause very different diseases. In terms of severity Singapore Haemorrhagic Fever stands between dengue fever and the haemorrhagic fevers of Thailand and the Philippines. We cannot say to what factor the difference in severity is due to. Perhaps it is due to a difference in the virulence of the viruses for which we have yet to develop a suitable virulence test.

Assuming that these variants in virulence exist, the question arises as to whether they had existed all the time or were only recently evolved. If the former is the case then with respect to Singapore Haemorrhagic Fever, the virus must have been imported and the problem is how this came about. A more attractive speculation however, is that the dengue viruses are subject to genetic variation like the influenza viruses and the haemorrhagic fevers are expressions of that variation brought to light because virologists are now studying these problems.

ACKNOWLEDGEMENTS

We wish to thank Mr. K. Kanapathipillai for his technical assistance and Mr. R. Manikam for the illustrations. The work was aided by a grant from the Rockefeller Foundation N. Y. and a grant from the George Williams Hooper Foundation, California.

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