

This paper reports studies on virological investigations of overlapping outbreaks of dengue and influenza in Vellore, India, in 1968. Activity of all four of the commercially recognized types of dengue virus is discussed.

THE 1968 OUTBREAK OF DENGUE IN VELLORE, SOUTHERN INDIA*

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Introduction

THOUGH dengue had been diagnosed clinically in Vellore for many years, scientific interest in the activities of its etiologic agent was aroused only in 1956 when type 2 virus was isolated fortuitously, and for the first time in Vellore, at the start of a pilot study orientated toward detection of infections with Japanese Encephalitis virus.^{1,2} Since then, proved endemic activity of dengue virus has become a matter of record, more frequently than not with demonstration of simultaneous activity of at least two of the recognized types. In 1968, strains of all four of the recognized types of dengue virus were isolated both from mosquitoes and man.³ Though, at the time, this was believed to be unique, it was found later that Russel, *et al.*, had also obtained evidence of simultaneous activity of the four types of dengue in 1967 in South Vietnam.⁴ Summarizing data related to more than 250 strains of virus recovered from specimens collected during the ten-year period, January

1959–December 1968, are shown graphically in Figure 1.

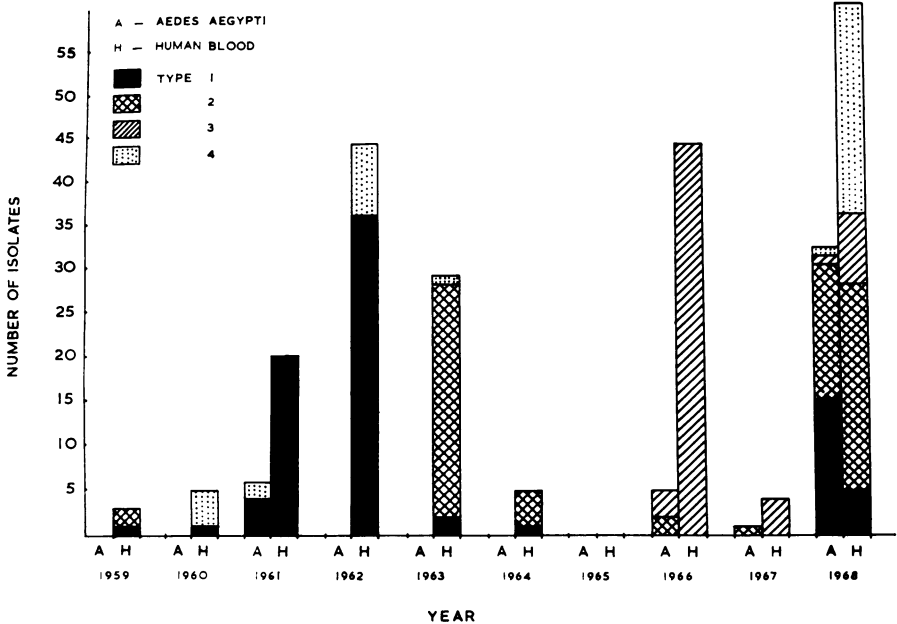
The purpose of this paper is to report further findings of studies on 1968 materials and single analyses of these with respect to certain factors of epidemiological concern, including the occurrence of an overlapping outbreak of influenza.

Materials and Methods

Reference may be made to the earlier report of the 1968 Vellore outbreak of dengue³ for details pertinent to the collection of materials and to the methods employed for laboratory studies. Briefly, the paired specimens of blood, collected for study, were obtained for the most part from patients who presented at the Staff-Student Health Clinic of the Christian Medical College Hospital (CMCH) with fever and signs suggestive of dengue or of influenza, an outbreak of which occurred also during the "dengue season," as mentioned above. Acute illness sera were stored at -50° C; convalescent and post-convalescent at -20° C. Virus isolation attempts were made after detection of arbovirus Group

* Composite report of isolation of four dengue types, with simple analyses related to certain epidemiological features, including an overlapping outbreak of influenza.

Figure 1—Dengue virus isolates, Vellore, 1959-1968



B antibody response in the patients concerned.

A total of 332 staff/student patients contributed paired sera between mid-July and the end of December. Of these, 47.3 per cent were resident on the midtown hospital compound, a few on the rurally located college campus about 4 miles away; 52.7 per cent resided in scattered areas of Vellore town and vicinity, over what in metropolitan terminology might be called "Greater Vellore." Isolation attempts were made on 106 specimens from patients on whom an arbovirus Group B antibody response was demonstrated during the dengue season, a few of these contributed by others than staff. Specimens from an additional 19 patients, who were seropositive, were not considered suitable for isolation attempts, usually because they had been obtained only after the patients had become afebrile. Very early in the season a few specimens from patients on whom convalescent speci-

mens could not be obtained were inoculated on receipt into mice or cell cultures, and virus was isolated from two of these.

Aedes aegypti for virological study were all collected in one section of the town, Saidapet, held for a minimum period of 48 hours at ambient temperature, and then divided into pools, of which there was a total of 103. Mouse inoculations were carried out usually on the day of receipt from the entomologists, rarely on the next day, in which latter case the pools were further stored at -50° C.

For various reasons (chiefly of economy) in attempts to isolate virus, infant mice, primary monkey kidney (PMK) and then BS-C-1 cell cultures were employed successively, and in accord with our routine procedures.^{5,6,7,8,9} For identification, complement fixation by infected mouse brain antigens in the presence of immune sera raised to Vellore prototypes of the four recognized

types of dengue virus, or neutralization in cell culture by known immune sera, also according to our routine procedures, was employed.

Tests for arbovirus hemagglutination-inhibiting (HI) antibody were carried out according to the procedures of Clarke and Casals,¹⁰ except that a microtechnic was used, with employment of Dengue type 2 and Japanese Encephalitis viruses initially, as "screening" antigens. Influenza HI tests were performed, likewise by microtechnic, with cell culture fluid as antigen, harvested from PMK cultures inoculated with a strain of Asian Influenza A₂, Hong Kong variant, which had been isolated in 1968 by Dr. N. Veeraraghavan, Director, Pasteur Institute, Coonoor. The sera were treated with potassium periodate and absorbed with goose blood cells, which cells were used also in the HI tests. Details of this procedure are to be published later with a full report and anal-

ysis of findings relevant to the outbreak of influenza.

Results

Dengue Antibody Response and Isolates

Reference to our earlier paper³ will show, as indicated above also, that Group B antibody response had been detected in about one-third of the 1968 febrile patients from whom paired sera were obtained. Analysis of serologic findings is presented later in this report. In our previous paper intimation was given of the recovery of 74 isolates of dengue virus, 43 from human blood, 31 from *Aedes aegypti*, including strains of all four types from both sources. Ultimately this total was increased to 92. It must be mentioned again that successive attempts had been made to isolate virus, first in mice, then in PMK cell cultures, and, finally, in BS-C-1 cell cultures. Results are shown in Table 1. It may be

Table 1—Summary of 1968 isolates and procedures by which they were recovered

Isolation system	Source of specimen	Number of strains and type of dengue isolated*				Total strains
		1	2	3	4	
Mice	<i>Aed. aegypti</i>	13	13		1	27
	Human blood	2	11		18	31
	Total	15	24		19	58
PMK†	<i>Aed. aegypti</i>	2	2	1		5
	Human blood	2	4	4	4	14
	Total	4	6	5	4	19
BS-C-1‡	<i>Aed. aegypti</i>					
	Human blood	1	8	4	2	15
	Total	1	8	4	2	15
Total	<i>Aed. aegypti</i>	15	15	1	1	32
	Human blood	5	23	8	24	60
	Total	20	38	9	25	92

* Sequential isolation attempts were made, first in Mice, then in Primary Monkey Kidney† Cell Cultures, and finally in BS-C-1‡ Continuous Line Grivet Monkey Kidney Cell Cultures.

Table 2—Dengue virus isolates, Vellore, July-December, 1968

(no)	Human blood												<i>Aedes aegypti</i>																	
	No. attempts				No. isolates				No. attempts				No. isolates				No. attempts				No. isolates									
	1		2		3		4		Total		1		2		3		4		Total		1		2		3		4		Total	
	No.	Type	No.	Type	No.	Type	No.	Type	No.	Type	No.	Type	No.	Type	No.	Type	No.	Type	No.	Type	No.	Type	No.	Type	No.	Type	No.	Type		
July	7	0	0	1	2	3	3	10	0	0	0	0	0	0	0	0	0	0	0	0	17	0	0	1	2	3				
Aug.	11	0	4	0	1	5	5	10	0	0	0	0	0	0	0	0	0	0	0	21	0	4	0	1	5					
Sep.	39	3	12	7	7	29	29	17	0	0	0	0	0	0	0	0	0	0	0	56	3	12	7	7	29					
Oct.	27	1	3	0	12	16	16	29	8	4	0	0	0	0	0	0	0	0	0	56	9	7	0	12	28					
Nov.	14	0	4	0	1	5	5	19	5	8	1	1	1	1	1	1	1	1	1	33	5	12	1	2	20					
Dec.	8	1	0	0	1	2	2	18	2	3	0	0	0	0	0	0	0	0	0	26	3	3	0	1	7					
Total	106	5	23	8	24	60	60	103	15	15	1	1	1	1	1	1	1	1	1	209	20	38	9	25	92					

seen that 58 of the 92 isolates, 63 per cent, were recovered in mice. Approximately a fifth of the strains, which could not be isolated and/or established in mice, were detected as interfering agents in PMK cultures. Another sixth, for isolation of which both mouse and PMK procedures have proved unsuccessful, were recovered in BS-C-1 cell cultures.

Summarizing data related to the source and month of collection of specimens and the type of virus isolated are presented in Table 2. Note that from human blood 60 dengue viruses were isolated, 5 type 1, 23 type 2, 8 type 3, 24 type 4; and from mosquitoes 32, 15 type 1, 15 type 2, and one each of types 3 and 4.

Chronology

As noted above, the 332 specimens of paired sera which had been obtained from staff and students of CMC & H, were found to be distributed almost equally among those who were resident on the hospital compound, or college campus, and those who resided in scattered areas of town. Though this distribution, as well as the large number of patients, was a reflection of the overlapping outbreaks of dengue and influenza, opportunity was thus afforded for study in a very limited way of the chronology and ecology of the dengue outbreak. See Figure 2 for a chronological picture of the outbreak of dengue, as indicated by weekly incidence of illness in patients who showed arbovirus Group B antibody response, and by isolation of virus from mosquitoes as well as from human blood. Note that incidence of illness appeared to be greater in hospital and college compound residents for July-mid-September, then greater for town residents for the next two months, and fairly similar for both groups after that. Note also that increase in the adult *Aedes aegypti* population in the Saidapet section of Vellore town seemed to correlate with increase in in-

idence of infection in town residents, as well as that of infection of the mosquitoes, as proved by isolation of virus

from human and arthropod specimens. Data relevant to the calculation of the *Aedes* densities are shown in Table 3.

Figure 2—Chronology of 1968 outbreak of dengue as reflected by examination of specimens obtained from affected staff and students of CMC & H and *aedes aegypti* density Saidapet, Vellore Town

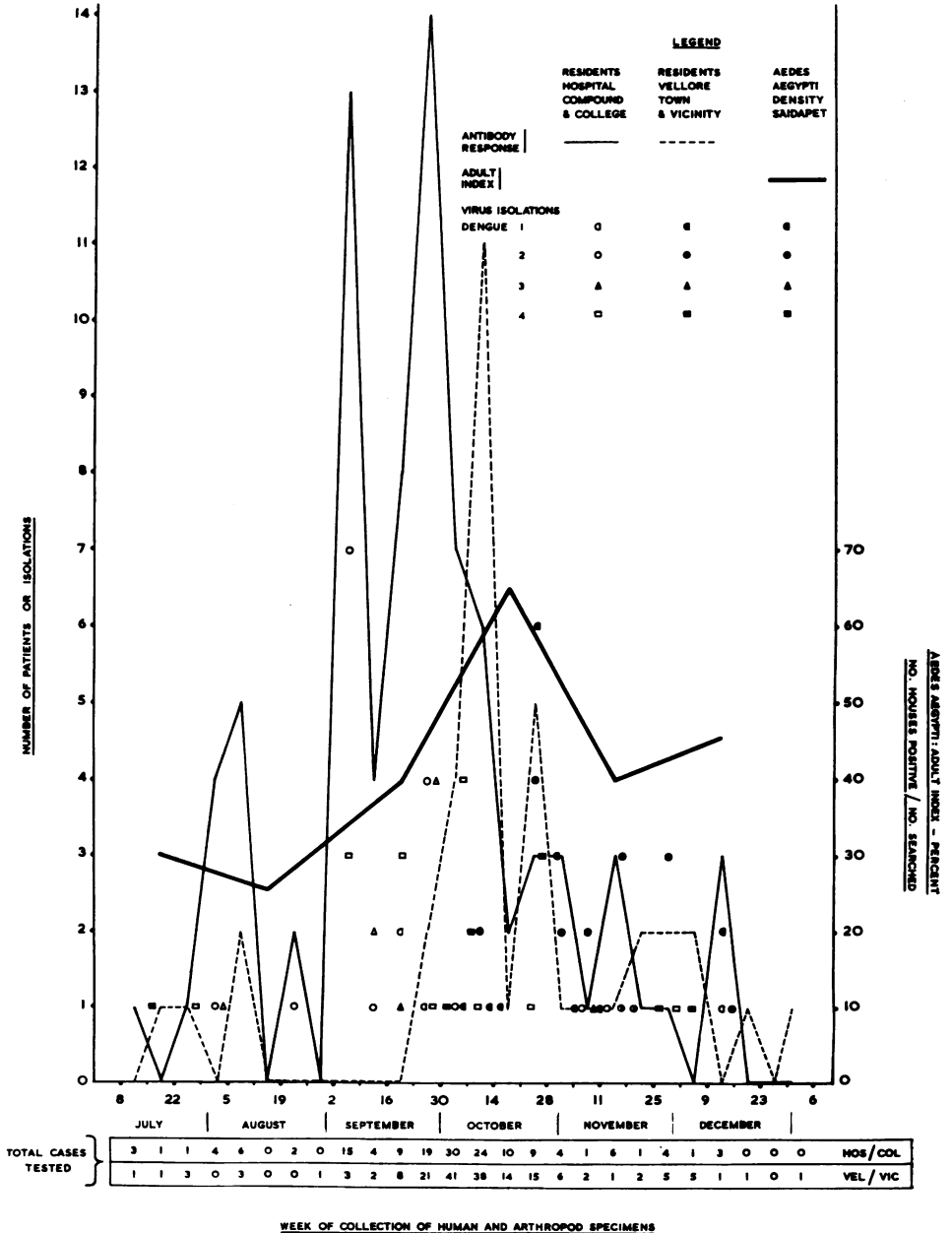


Table 3—*Aedes aegypti* survey, Saidapet, July-December, 1968

(mo)	No. of collections	No. of manhours	No. of female <i>A. aegypti</i>	Av. no. of <i>A. aegypti</i> per man hr.	No. of houses searched	No. of houses positive	Adult index per cent	Av. no. of <i>A. aegypti</i> per house
July	8	67.5	361	5.3	336	102	30.3	1.1
Aug.	9	92.5	331	3.6	414	106	25.6	0.8
Sept.	9	62.5	441	7.1	368	147	39.9	1.2
Oct.	8	50.0	921	18.4	280	182	65.0	3.3
Nov.	9	85.0	739	8.7	370	148	40.0	2.0
Dec.	9	105.0	802	7.6	478	218	45.6	1.7

Antibody Status at Time of Illness

Tabulation of patients according to whether or not arbovirus Group B antibody had been detected in acute illness sera revealed evidence of probable prior experience with these viruses in about 80 per cent of the town residents, in contrast to 46 per cent of the hospital and college compound residents. It should be noted that many of the latter had come from places outside of Vellore for undergraduate and postgraduate medical studies or studies in the paramedical fields. A few of the town residents were also CMC students new to Vellore who were living extramurally; the majority, however, had lived in Vellore town and vicinity for years, many from birth. It is acknowledged that in some instances the antibody detected in the acute illness sera may have been formed only in response to the current episodes of infection. No attempt was made to analyze the antibody, and even the lowest level of antibody detected was regarded for the purpose of these analyses as evidence of prior encounter with Group B arboviruses. Most of the acute illness specimens were collected within 3 days of onset of fever.

See Figure 3 and note that among the 138 town residents for whom there was evidence of previous encounter with viruses of the dengue group, there were 25, or 18 per cent, whose 1968 illnesses were diagnosed serologically as cases of arbovirus Group B infection; similarly, among the 72 hospital/college compound residents, there were 34, or 47 per cent. Among the town residents for whom prior experience with Group B arboviruses was *not* detected, sero-diagnosis of dengue was positive in 1968 for 35 per cent; among the comparable hospital/college group, 56 per cent. Thirteen strains of virus were recovered from town residents, 5 from persons whose acute illness sera contained antibody. Forty-four strains of dengue were iso-

lated from staff and students resident on the hospital compound or at college, 16 from individuals in whose acute illness specimens HI antibody was detected. See Table 4.

Overlapping Influenza

Serodiagnosis of influenza was made for 212, or 63 per cent, of the 332 patients who contributed paired sera. See Figure 4 and note that the peak incidence of influenza among the town residents occurred in October, at the time that they were affected mostly by dengue also. The highest incidence of influenza among hospital/college residents occurred likewise in October; but dengue incidence was highest for this group in September. In 49, about 15 per cent, of the patients, infections with both influenza and dengue viruses must have occurred at the same time, or within a very short space of time, since HI antibody responses were detected to both. Most of the convalescent specimens were collected 10 days after receipt of the acute

illness specimens, some 3 weeks afterwards, only a few after a longer interval.

Viruses comprising all four of the recognized types of dengue were isolated from 17 of the patients with antibody responses both to dengue and influenza. These included one strain of Dengue type 4 isolated from a patient in whom hemorrhagic phenomena had been observed.¹¹ In 15 per cent of the patients HI antibody was not detected to either dengue or influenza.

Dengue HI Antibody Patterns

For some time we have been interested in the patterns of persisting antibody following primary and sequential dengue infections;^{8,9} and it had been found that homologous antibody to the type of dengue first encountered was most likely to persist in highest titer. Among the 1968 patients, 56 who had apparently suffered primary attacks, as judged by the results of the HI screen tests, in which Dengue type 2 and Japanese Encephalitis antigens had been

Figure 3—Incidence of dengue in staff residing in different localities, 1968

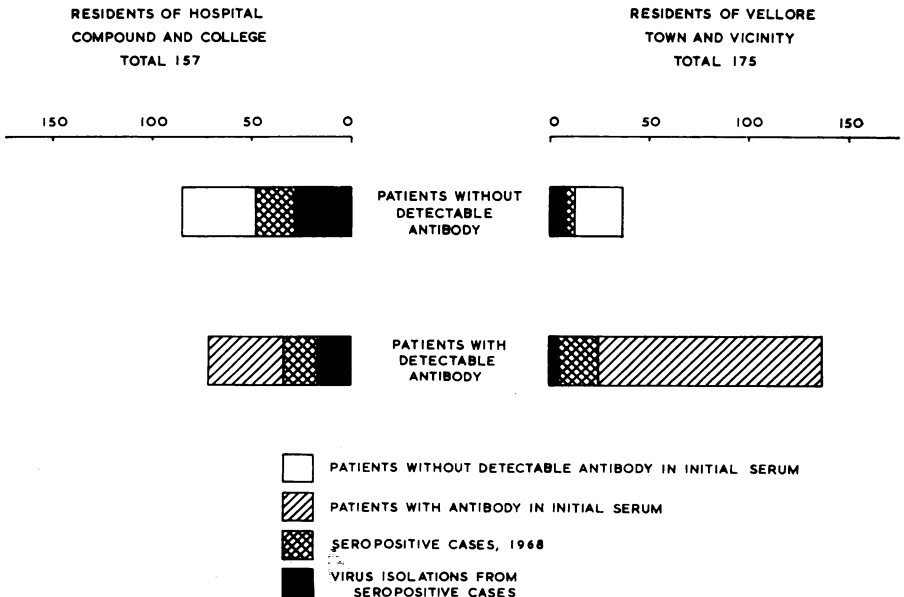


Table 4—Antibody responses and dengue virus isolations from hospital/college and Vellore town/vicinity patients according to presence or absence of detectable arbovirus group B antibody in initial specimens

Group	No antibody in initial serum				Antibody in initial serum				Total cases tested					
	Antibody response, 1968		No antibody response, 1968		Antibody response, 1968		No antibody response, 1968		Antibody response, 1968		No antibody response, 1968			
	Isolate	No isolate	Total	No antibody response, 1968	Isolate	No isolate	Total	No antibody response, 1968	Isolate	No isolate	Total	No antibody response, 1968		
Hospital/college	28	20	48	37	85	16	18	34	38	44	38	82	75	157
Vellore town/vicinity	8	5	13	24	37	5	20	25	113	13	25	38	137	175
Total cases	36	25	61	61	122	21	38	59	151	57	63	120	212	332

used, contributed post-convalescent as well as the routine convalescent specimens. Subsequently, when tests of the sets of three specimens were carried out with antigens to the four types of dengue, it was found that 7 of these subjects had probably had a previous attack of dengue; low levels of antibody to type 1, 3 or 4 were found. Among the remaining 49, virus isolations from 30 proved that dengue infections had occurred in 1968 with one or another of the four recognized types of virus. In about 40 per cent of these, post-convalescent titers were highest for the type of virus which had been isolated, a figure somewhat lower than that obtained earlier. See Table 5.

It was of interest to find among the 7 cases excluded at the time of analysis, as mentioned above, 6 patients with post-convalescent dengue antibody at highest titer following their 1968 illnesses to the same type found in their 1968 acute illness sera.

Discussion

The opportunities for study which were afforded by the nature of the outbreak of febrile illness among staff and students of the CMC & H during the latter half of 1968 were unique in our experience, in that for the first time simultaneous activity of all four of the commonly recognized types of dengue was detected

Figure 4—Overlapping outbreaks of dengue and influenza in 1968 among staff and students of CMC & hospital serologically diagnosed cases

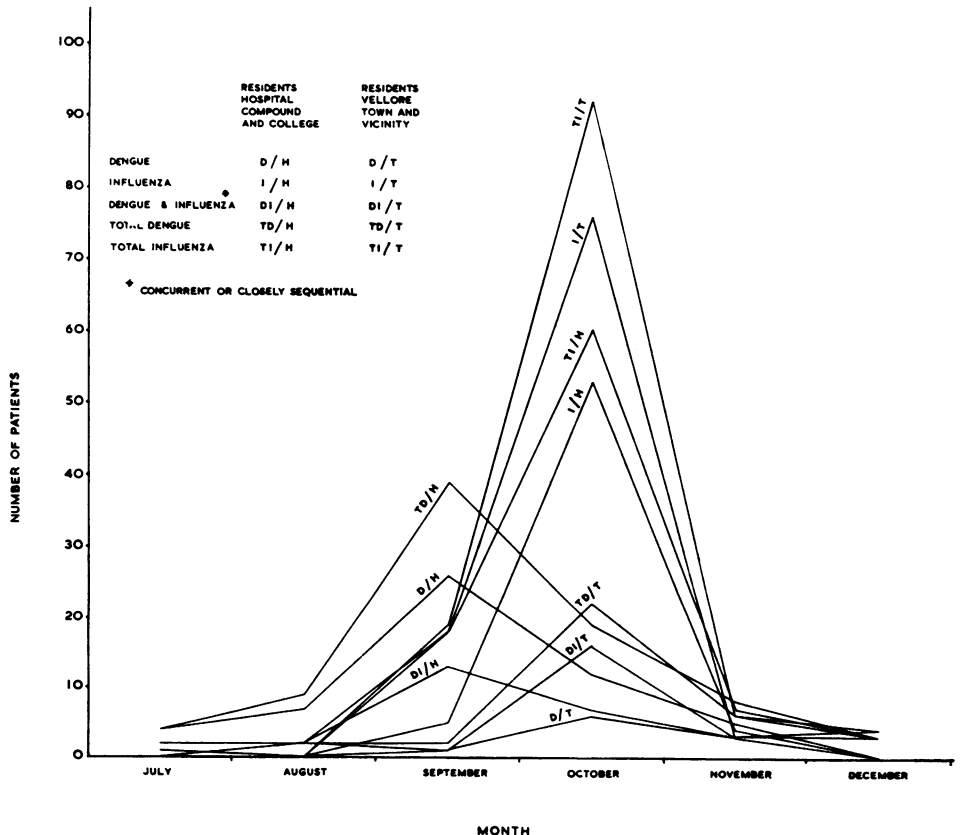


Table 5—Predominant post-convalescent antibody as related to dengue viruses isolated from apparently primary episodes of dengue-like illness, 1968 cases

Virus isolated	Post-convalescent antibody of highest titer					
	D1*	D2	D3	D4	Equivocal	Total
Nil	1	1	5	6	6	19
D1				1	2	3
D2	1	2	2	5	4	14
D3		1			1	2
D4			1	10		11
Total	2	4	8	22	13	49

* D = Dengue; figures refer to type.

early in the course of the outbreak; and later in the course, concurrent activity of influenza. Dengue viruses were isolated from human blood and from pools of *Aedes aegypti* in our laboratories. A strain of Asian Influenza A₂, Hong Kong variant, was isolated in the laboratories of the Pasteur Institute, Coonoor, from throat-washings of a patient in Vellore from whom, along with a few others, Dr. N. Veeraraghavan, Director of Pasteur Institute, had collected specimens soon after he had collected specimens from patients in Madras (Personal Communication).

During the 10-year period January 1959–December 1968, a total of 258 strains of dengue virus was isolated, over a third of these from specimens collected in 1968. Cell culture procedures were not employed routinely until 1966; and studies of specimens collected in that year indicated that the BS-C-1 continuous grivet monkey kidney cell line was much more susceptible than infant mice to Dengue type 3, strains of which were encountered in Vellore for the first time in 1966.⁹

For reasons of economy in study of 1968 materials, mice were first employed as described above, then PMK cultures and finally BS-C-1 cultures, in successive attempts to isolate viruses. Though isolation rates that could be compared with each other were not obtained thereby,

it seems obvious that of the three, BS-C-1 procedures would be preferable if a choice could be made: 16 per cent of the 1968 viruses were recovered in BS-C-1 cultures after attempts to isolate agents in mice and PMK cultures had been unsuccessful. It may be noted in Table 1 that mouse procedures did not yield any of the type 3 strains; further that PMK cultures yielded strains of all types and that BS-C-1 cultures yielded strains of all types in the absence of recovery of agents in mice and PMK cultures inoculated sequentially with aliquots of the same specimens.

Through the years the majority of cases of dengue referred to us for virological investigation had occurred in staff/students and for the most part in those new to Vellore and resident on the hospital compound. The overlapping outbreak of influenza in 1968 resulted in the accumulation of specimens from a larger number of staff/student patients who were not resident on the hospital compound. *In toto*, 120 of the 332 patients investigated in 1968 showed arbovirus Group B antibody response, a 36 per cent incidence. When analysis revealed that there were almost equal numbers in the two groups of patients, it was decided to compare them in various ways. Thus, it was found from serologic tests that 138 of the 175 town/vicinity residents had had, in all probability,

prior encounter with dengue virus, in contrast to 72 of the 157 hospital/college residents, 80 per cent in contrast to 46 per cent. Furthermore, the incidence of arbovirus infections in 1968 was lower for town than for hospital residents, and the number of isolates from the former was less. Thus it would appear that the town residents enjoyed a degree of protection in 1968, possibly because of repeated encounters with the dengue viruses, or even because of the widespread outbreak of dengue in 1966 when activity of type 3 in Vellore was recognized for the first time, with indication that town as well as hospital compound residents were affected.^{9,12} It is likely also that the dengue, perhaps mild in nature, which was detected among town residents would have been lower still in incidence but for the overlapping outbreak of influenza. Resident staff and students were more prone to report to the clinic and to be admitted to the hospital (at which times, usually, the acute illness specimens were obtained) in order to have care which could not be provided in their hostels. On the other hand, non-resident staff often failed even to report to the clinic; and frequently, though they did report, they were asked to remain at home and receive care from their families. In the latter event specimens were collected before they left the clinic.

It was found from chronologically plotted data that dengue among the resident staff/students had preceded that among the non-resident; and that the increase of adult *Aedes aegypti* population and infection of the mosquitoes collected from one section of Vellore town, coincided with increase of dengue infection among the staff/student population who lived in Vellore town and vicinity.

It had occurred to us that the rapid spread of influenza virus among our clientele might have affected the course of the dengue outbreak. From prelim-

inary tabulation of total figures it appeared that interference may have occurred, in that the overall incidence of Group B arbovirus infection dropped as the incidence of influenza increased. When, however, the findings of serologic tests were analyzed according to the place of residence of the patients, it became evident that no such phenomenon had occurred. Though influenza followed dengue on the hospital/college compounds, the two coincided in the town. Overall findings indicated that among the 332 patients, 71 suffered from dengue only, 163 from influenza only, 49 from both dengue and influenza either concurrently or sequentially, and 49 from neither of these. Dengue viruses of one or another of all types were isolated from 17 patients who showed the dual antibody response; a strain of type 4 from one patient whose illness conformed with the syndrome of hemorrhagic dengue. Whether or not influenza infection contributed in any way to the hemorrhagic phenomena observed is not known.

Finally, some mention must be made of the patterns of persisting dengue antibodies, in which we have been interested for some time.^{8,9} As described above, routine HI tests for detection of arbovirus Group B antibody responses following the 1968 episodes of illness were performed with Dengue type 2 and Japanese Encephalitis viruses as "screening antigens." When HI tests were carried out with antigens to the four recognized dengue sero-types on sets of three specimens—acute, convalescent, post-convalescent—from 56 patients in whom the lack of detectable D2 and JE antibody had been presumed to indicate no prior contact with arboviruses of Group B, results were not as clear-cut as had been expected. Still there was evidence of persistence of homologous antibody in highest titer in 12 of 40 patients with proved viremia, a confirmation of previous findings, though lower in incidence.

Whether or not patterns may have been altered by the simultaneous activity of the four types of dengue virus, possibly even concurrent infection with more than one type, is not known. Strain differences may also have played a role: the antigens employed were prepared from Vellore prototype strains, isolated early in our studies. It is in fact suspected that such differences may come to light when it is possible to carry out precipitin-in-gel tests such as those performed by Ibrahim and Hammon¹³ during their studies of dengue viruses isolated in Thailand in 1958 and recognized then as being related to types 1 and 2.^{14,15} Further studies had revealed differences sufficient in degree to suggest the possibility that the Thai strains might belong to two additional serotypes, 5 and 6¹⁶ which differences have been confirmed more recently by the precipitin-in-gel tests mentioned above.

Summary

A total of 332 CMC & H staff/student patients contributed paired sera between mid-July and the end of December, 1968, for virological studies related to overlapping outbreaks of dengue and influenza. Nearly half of this group were resident on the hospital compound, a few on the college campus. The others resided in scattered areas of Vellore town and vicinity.

Antibody response to Group B arboviruses was detected for about one-third of the patients. Isolation attempts were made on 106 of the acute illness blood specimens collected in 1968; and 60 dengue viruses were recovered: 5 type 1, 23 type 2, 8 type 3, 24 type 4.

During the same period 103 pools of *Aedes aegypti* were collected in Saidapet, a section of Vellore town, for virological studies. Virus was isolated from 32: 15 each of Dengue types 1 and 2, one each of types 3 and 4.

About 65 per cent of the isolates were recovered in mice, 21 per cent in pri-

mary monkey kidney cell cultures and 16 per cent in BS-C-1 cultures, in successive isolation attempts beginning with the mouse procedures.

Analysis of findings with respect to dengue revealed that affected staff/student patients who were resident at hospital or college suffered peak incidence in September, whereas those who resided in scattered areas of Vellore town and vicinity were affected in highest proportion in October. Population densities of *Aedes aegypti* which were determined in one section of the town, and the number of viruses isolated from pools of the mosquitoes were both maximum in October, a correlation to incidence of infection in town residents.

Comparison of findings related to arbovirus Group B antibody status at the time of illness showed that about 80 per cent of the town residents had in all probability had prior contact with these agents in contrast to 46 per cent of the hospital/college residents. The incidences of dengue in both groups and the numbers of viruses isolated differed in such a way as to suggest that town residents had enjoyed a degree of protection against dengue in 1968.

From studies on sets of three specimens—acute illness, convalescent and post-convalescent—further evidence was obtained to support findings of earlier studies that indicated persistence of highest levels of antibody to the type of dengue virus encountered at the time of primary experience with the group.

Analyses of the results of influenza serologic tests revealed peak incidences of this infection in October for both groups of staff/students.

Among the 332 patients who had contributed two or more specimens of blood for laboratory studies, tests for hemagglutination-inhibiting antibodies showed that 71 had suffered from dengue only, 163 from influenza only, 49 from both dengue and influenza, either concurrently or sequentially, and 49 from neither of these.

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