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Prevalence of Dengue Infections in India

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Abstract. Infections attributable to dengue viruses have been frequently occurring in India, as also in South East Asian countries. Benign clinical manifestations, have been rampant in this country. Various aspects of dengue infections in the context of India e.g., occurrence, clinical profile, viral isolations, serological surveys, pathogenicity and vector ecology have been discussed in the present review.

1. Introduction

The outbreaks caused by mosquito borne dengue viruses are prevalent in tropical and subtropical countries of the world. Dengue infections have been rampant in India for the last two decades. With the advent of methods for viral isolations and serological techniques in India all the four serotypes (DEN 1 to DEN 4) of dengue virus complex have been extensively reported since 1956 from various places of the country. These outbreaks have assumed significance on account of their severity, frequency of occurrence and clinical profile.

Prior to world war II, the disease caused by dengue viruses were reported exclusively on the basis of typical clinical symptoms[1-5]. The aetiology of the viruses (DEN 1 and DEN 2) was first reported by Sabin[6] during an outbreak in Calcutta.

Serological surveillance against dengue viruses in India and the ecology of the vector have been briefly reviewed in the past[7-9]. In the present paper, the occurrence of these epidemics as evidenced by viral isolations, serological surveys, clinical features of the disease, mechanism of pathogenicity and the role of the vector *Aedes aegypti* as related to India have been reviewed.

2. Epidemiology

Dengue viruses are disseminated in nature simply by a man-mosquito-man cycle. The domestic mosquito *Ae. aegypti* is the principal vector of the disease. No extra human reservoir is required for the maintenance of these viruses in the environment. The vector thrives in urban and semiurban localities congested with human population. The mosquito breeds usually during rains or in any water logged containers. The disease has usually affected malnourished persons specially males. For these reasons the epidemics of dengue infections occurred in the congested urban and semiurban

places in India. Apart from this Halstead[10] visualized that native immunity in addition to host factors may determine the severity of epidemic.

Epidemics in India

Dengue outbreaks in India have been presented[11–42] in Table 1 in a chronological order. These viruses have been persisting in India year after year since 1956 when their aetiology was first established by isolation of DEN 2 from the serum of a six year old child at Vellore. Subsequently DEN 1 and DEN 4 were isolated[11,12] again at Vellore and DEN 3 at Madras[24]. In India the disease caused by dengue viruses, by and large manifested benign symptoms of the disease. In some places as in Calcutta[13–18], Vishakapatnam[19–20], Asansol[28] and Kanpur[31–33] (Dengue Haemorrhagic Fever) DHF had been an important clinical event.

Table 1. Incidence of dengue infections in India

Locality	Year and month of occurrence	Dengue serotype incriminated	Reference
Vellore	1956–1960 (Oct-Nov)	DEN 1, 2	11–12
Vellore	1961 (Sep-Nov)	DEN 4	11
Calcutta*	1963 (Jul-Mar)*1	DEN 2	13–18
Vishakapatnam	1964 (Jul-Sep)	DEN 2	19–20
Vellore*	1964 (Aug-Nov)	DEN 2	21–22
Nagpur*	1965 (Apr-Jun)	DEN 4	23
Madras	1965 (Oct)	DEN 3	24
Jabalpur	1966 (Aug-Sep)	DEN 3	25–26
Vellore	1966	DEN 3	27
Asansol	1967 (Jul-Oct)	DEN 2, 4	28
Delhi	1967 (Sep-Oct)	DEN 2	29
Vellore	1968 (Jul-Sep)	DEN 1, 2, 3 & 4	30
Kanpur	1968 (Aug-Oct)	DEN 4	31–32
Kanpur	1969 (Sep-Nov)	DEN 4 & 2	33
Ajmer	1969 (Aug-Nov)	DEN 1 & 3	34
Gwalior	1970 (Sep-Oct)	DEN 3	35
Bangalore	1970-71 (Nov-Mar)	DEN 1 & 2	36
Delhi	1970 (Oct)	DEN 2	37
Hardoi	1970 (Sep-Oct)	DEN 2	38
Jaipui	1971 (Aug-Nov)	DEN 1 & 2	39
Jaipur	1973 (Oct-Nov)	DEN	40
Jammu*	1974 (Aug-Sep)	DEN 2	41
Trichur	1974 (Aug-Oct)	DEN 2	42

*Other aetiological agents, specially Chikungunya virus concurrently existing.
*1Jul-Sep dominated by DEN 2 phase, and by Chikungunya virus thereafter.

Epidemic season

An evident dengue infections were generally encountered in India during or after rains, as an outcome of rise in vector population. The febrile phase normally commenced during July or August and perpetuated till September or October. Highest number of epidemics occurred in the month of September and lowest between December and June (Fig. 1). An exceptionally long-epidemic period was recorded in Calcutta

where the haemorrhagic fever continued from July to March. Dengue haemorrhagic fever was induced by Group B Flavivirus DEN 2 in July which remained viable among the population specially children until October. The next phase in continuation was induced by Group A Flavivirus-Chikungunya which persisted till March.

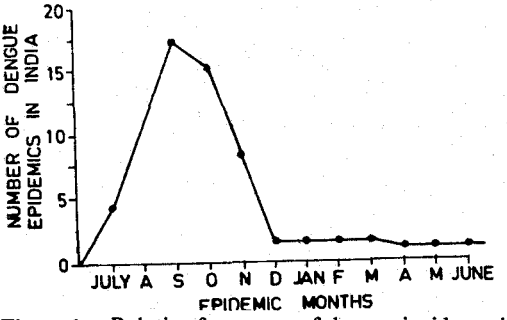

Figure 1. Relative frequency of dengue incidence in India to epidemic months.

The epidemic season deviated from the normal in Nagpur[23] and Bangalore[36]. One of the possible reasons for the seasonal aberration in Nagpur and Bangalore could be the fluctuating breeding habit of *Ae. aegypti* in different types of containers e.g. dumped and moist tyres or earthen-wares used for storing water during summers[23,43].

Age susceptibility

In general persons of all age groups sustained dengue infections in India. Except Calcutta epidemic, children below ten years were not usually affected during dengue outbreaks in India. The fatality rate was however reported to be relatively higher among children below this age especially on account of severe syndromes. Lack of immunity among children could be the possible reason for the high fatality rate. Calcutta HF (1963) epidemic can be compared to some South-East Asian epidemics at Manila[44] and Bangkok[45] with regard to age susceptibility.

The difference in the clinical manifestations of the disease was elicited in two different age groups during recurring dengue cases in Vellore[46]. Children below the age of six years indicated respiratory distress, whereas those above this age presented symptoms of classical dengue infection. Persons between 10 and 30 age group were in general susceptible to benign dengue infections. A wider susceptible age range of 5 to 60 years was however, noted in Asansol[28]. Healthy persons between 12 and 30 years were affected by the infection at Vishakapatnam[19,20], which was non-fatal though widespread and in this respect resembled Singapore[47] HF.

3. Clinical Features

Benign form of dengue infections dominated the Indian epidemic scene, as evident from overall clinical profile. Typical clinical symptoms noted during outbreaks in India were; pyrexia of 3 to 5 days duration which at times prolonged to 10 days, with remission of fever in between "Saddle Back Temperature Chart", Gastro Intestinal (GI) tract disorders e.g. epigastric discomforts, diarrhoea in majority of the cases and constipation in some cases. Myalgia, which is a characteristic symptom of the

disease, giving it its popular name of "Break bone fever" existed in mojority of the epidemics. However, artharlgia—a prominent feature of Chikungunya infection was noticed during dengue epidemic in Jabalpur[25]. Rashes were also present though infrequently, only in the form of maculopapular or at times measly rashes as in Ajmer[34].

Dengue haemorrhagic fever

DHF was not a prominent syndrome in India unlike South-East Asian countries, except in places depicted in Table 2. Calcutta[13–16] HF is a classical example of DHF in the context of India. Two aetiological agents involved in the epidemic show different manifestations of haemorrhagic syndromes. During dengue phase the haemorrhagic syndrome was well marked, which considerably declined in the later phase involving Chikungunya virus. In Vishakapatnam[20] a non fatal HF was recorded in 25.1 per cent young adults. Haemorrhagic and shock syndromes were present though insignificantly in Asansol[28] and Kanpur[32]. DEN 4 virus was recovered from a patient with DHF at Vellore[48]. A few cases with involvement of Central Nervous System (CNS) which is usually spared by dengue viruses, were reported from Ajmer[34].

Table 2. Dengue Haemorrhagic Fever (DHF) and Shock Syndrome (DSS) encountered in India

Place of occurrence	Year	Dengue serotype incriminated	Remarks
Calcutta	1963	DEN 2	Children below ten years main victims. Neurological complication in four reported cases attributed to chikungunya virus.
Vishakapatnam	1964	DEN 2	Mild haem manifestation with no mortality.
Asansol	1967	DEN 2, 4	Indication of shock syndrome in a male of 25 years. DEN 2 virus isolated, from the serum.
Kanpur	1968	DEN 4	DEN 4 isolated from 2 patients with indication of DSS.
Vellore	1968	DEN 1, 2, 3 & 4	DEN 4 isolated from a patient with DHF.
Ajmer	1969	DEN 1, 3	Neurological complications in some cases. Two cases revealed encephalitic syndrome. DEN 3 virus recovered from one of the sera.
Kanpur	1969	DEN 2	—

4. Viral Isolations

During outbreaks of dengue infections in India, dengue serotypes were frequently isolated both from human sera and mosquito pools.

Viral isolations from human sera

Data on the isolation of dengue serotypes (DEN 1–DEN 4) from the places of epidemics in India, as also the techniques employed for their isolation, are presented in Table 3. The most common method initially used for virus isolation was the intracerebral (at times intraperitoneal or subcutaneous) inoculation of suckling infant

mice or adult Swiss albino mice followed by serologic confirmation by various procedures. Halstead[49] also elucidated that the viral factor isolated from DHF/DSS patients should be substantiated by reisolation from "separate aliquots of original material" to confirm the exact aetiology. However, the isolation of dengue viruses by mouse inoculation was found to be difficult. It has been observed that these viruses have poor adaptability in laboratory animals and even in mice they require several passages for adaptations[50].

Table 3. Isolation of dengue serotypes (DEN 1–4) from human sera during incidence of dengue infections in India

Place of isolation	Year	No of serum samples attempted	Method used for isolation	No. of viral isolations			
				DEN 1	DEN 2	DEN 3	DEN 4
Vellore	1956	—	SM, SAM	—	3	—	—
Vellore	1959	—	SM, SAM	1	2	—	—
Vellore	1960	—	SM	1	—	—	4
Vellore	1961	57	SM	20	—	—	—
Vellore	1962	108	SM	36	—	—	8
Vellore	1963	77	SM	2	26	—	1
Vellore	1964*	315	SM	1	4	—	—
Vellore	1966	130	SM, BSC-1	—	—	40	—
Vellore	1967	—	—	—	—	4	—
Vellore	1968	393	SM, MKEC & BSC-1	4	15	4	20
Calcutta	1963*	222	SM	—	1	—	—
Vishakapatnam	1964	19	MKEC	—	4	—	—
Madras	1965	4	SM, MKEC & BSC-1	—	—	2	—
Jabalpur	1966	69	BSC-1	—	—	4	—
Asansol	1967	77	SM	—	7	—	3
Delhi	1967	20	SM, BHK-21, Vero	—	9	—	—
Kanpur	1968	224	SM	—	—	—	2
Kanpur	1969	48	SM	—	2	—	6
Ajmer	1969	83	SM, ATC-15, Vero	15	—	15	—
Hardoi	1970	40	SM	—	2	—	—
Jaipur	1971	103	SM, AACC	12	3	—	—
Bangalore	1970–71	47	SM	1	2	—	—
Jammu	1974	143	SM, Vero	—	3	—	—
Trichur	1974	31	SAM	—	1	—	—

*Chikungunya viral isolates outnumbered dengue serotype(s) isolates.

Abbreviation : SM = Inoculation in suckling mice.
SAM = Inoculation in Swiss albino mice.
BSC-1 = Renal epithelial cell line from African green monkey kidney.
Vero = Vero cell line.
MKEC = Epithelial cell line derived from Indian bonnet monkey (*Macaca radiata*).
ATC-15 = *Aedes albopictus* cell line.
AACC = *A. albopictus* primary cell culture.
BHK-21 = Baby hamster kidney cells.

Tissue culture systems in addition to mouse inoculation were subsequently applied with success. Tissue culture systems used for isolation of dengue viruses in India were; (i) Vero cell line[29,34,41,51], (ii) Baby Hamster Kidney cell culture[29] (BHK-21), (iii) Continuous cell line derived from African green monkey kidney cells (BSC-1)[24,26,27,30], (iv) Epithelial cell line derived from Indian bonnet monkey; Macaca Radiata (MKEC)[52], (DEN 2 and DEN 4 viruses were adapted to MKEC cell line during outbreak at Vishakapatnam[53]), and (v) *Aedes albopictus* cell line (ATC-15)[31] and primary culture[39]. All the four serotypes were isolated in ATC-15 cell line producing characteristic Cytopathic Effect (CPE)[54].

Circulating immune complexes, early development of antibodies and rapid neutralization of the virus[55] impede the recovery of dengue viruses from DHF/DSS patients. It was evident from Calcutta[56] HF and Kanpur[32] HF epidemics, where out of 222 and 224 sera respectively, only 1 strain of DEN 2 and 2 strains of DEN 4 viruses were isolated.

Only one of the serotypes was involved in some places of dengue infections except DEN 1 virus, whereas more than one were incriminated for the dissemination of the infections at other places. In Vellore all the four serotypes were present during the epidemic (Table 1).

Viral isolation from mosquito pools

Dengue serotypes isolated from the pools of *Ae. aegypti* during dengue outbreaks in India are reported in Table 4. All the four serotypes were isolated from *Ae. aegypti* pools in India. Carey *et al.*[57] were the first to report the isolation of DEN 1 and DEN 4 viruses from female *Ae. aegypti* pools from Vellore. DEN 2 and DEN 3 viruses were subsequently isolated from mosquito pools in Vellore[27] and Madras[26] respectively. All the four serotypes were isolated from mosquito pools in Vellore[30] epidemic. It was noted that mosquito density enhanced during outbreaks. Quantitatively the highest number of strains of DEN 2 virus were isolated followed by DEN 1 and then an equal number of DEN 3 and DEN 4 isolates.

Table 4. Isolation of dengue serotypes (1–4) from mosquito pools (*Aedes aegypti*) in India

Place of isolation	Year	No. of viral isolations				Reference
		DEN 1	DEN 2	DEN 3	DEN 4	
Vellore	1961	4	—	—	2	56
Vellore	1966	—	2	3	—	27
Vellore	1967	—	1	—	—	30
Vellore	1968	15	14	1	1	30
Madras	1965	—	—	1	—	26
Nagpur	1965	—	—	—	3	22
Delhi	1967	—	1	—	—	29
Ajmer	1969	3	—	1	—	34
Bangalore	1970-71	7	22	—	—	36
Jaipur	1971	2	1	—	—	39

5. Serological Surveillance

Haemagglutination Inhibition (HI) was frequently used to detect anti dengue antibodies followed by Complement Fixation Test (CFT) and Neutralization Test (NT).

During the first extensive serological survey in India evidence for antibodies against DEN 1 and DEN 2 serotypes were found respectivelv in 40 per cent and 20 per cent of 588 serum samples collected from 38 different endemic areas[58]. The results indicated elevated immunity level in Broach (Gujrat) and Nagpur. Review on subsequent serological survey in India[9] indicated endemicity to DEN 1 virus in Calcutta, Jamshedpur and Indore. At Ramtek (Nagpur), children below six were found serologically positive. Rural Maharashtra lacked antibodies to dengue viruses among persons of 5 to 50 years of age. Out of 700 sera collected at Jammu and Kashmir state none was found to be positive. Survey at Pune revealed West Nile overlapping dengue in endemicity. In another exhaustive report on serological survey in South India[59] against Group B Arboviruses, East and West coast sectors showed varied DEN 2 virus activity. Kerala state and South Kanara districts revealed low activity of dengue viruses (DEN 2 & DEN 1) as compared to Tamil Nadu. This survey also recorded significantly higher distribution of antibodies among Urban population. Calcutta city, adjoining villages and Darjeeling were found to be highly endemic to dengue[60]. Antibodies to DEN 2 virus were further detected in 170 of 211 sera tested in Calcutta city[61]. Prevalence of DEN 2 antibodies was noted in Northern Assam and Arunachal Pradesh[62]. In a survey at Lucknow, HI antibodies to dengue viruses; DEN 4, DEN 2, and DEN 1 were found in 68 per cent, 66 per cent and 56 per cent of tested sera[63]. In Rajasthan seven ecologically distinct areas were reported to be endemic to dengue viruses[64].

From the studies on extensive serological survey in India it is explicit that antibodies against DEN 2 and DEN 1 viruses are comparatively more pronounced among urban population than the other two serotypes.

6. Mechanism of Pathogenicity

Halstead *et al.*[65,66] elucidated that a secondary infection with a heterologous dengue serotype may induce hypersensitivity resulting in DHF. This view was amply supported by other workers[67,68]. Despite common antigens, each one of the four serotypes possesses a specific antigenic component. It may therefore be expected that the initial impact of primary dengue infection results into sensitization of immune system and the subsequent infection with a heterologous serotype may cause DHF/DSS.

Immunoglobulin (Ig) response to a primary dengue infection with a given serotype enhances IgM level. IgG (anti dengue antibodies) appears much later in the immunological process. The chances of formation of immune complexes during primary infection are evidently remote. However, on a secondary exposure of the same individual to a heterologous serotype, IgG level is considerably elevated resulting in almost complete depletion of IgM from the system. However, Scott *et al.*[69] reported that during Bangkok epidemic, children exhibited DSS even on the primary exposure to dengue infection.

Immune complexes

Complement components level falls appreciably during shock[55,67,70–71]. Russel & Brandt[55] found that virion and IgG exist concurrently in the blood, forming immune complexes. The formation of immune complexes and the resultant dramatic fall of component 3 (C3) clinically manifest shock syndrome. It has been elucidated that two of the three antigens of DEN 2 virus; rapid and slow sedimenting haemagglutination antigens (RHA-SHA) take part in the formation of immune complexes[71]. DSS was found to occur on maximal production of these complexes[72]. Elevated IgE levels were reported in a retrospective study of sera collected from Bangkok DHF patients. Enhanced IgE eventually promotes histamine production and consequent vascular permeability and vascular collapse[73]. The double aetiology hypothesis[74] advanced recently to explain DHF, also finds strength in IgE mediated histamine production. This view however accounts for only abdominal haemorrhages.

Chikungunya, a Group A Arbovirus, in conjunction with other dengue serotypes may provoke DHF/DSS as evidenced during epidemics in Manila, Penang, Bangkok, Vietnam[49] and Culcutta[13–18]. Based on their study of Bangkok epidemic of 1960 Hammon and Sather[45] postulated that severe syndromes are the cumulative effect of all the four serotypes. However in India even the existence of all the four serotypes in Vellore outbreak[14] failed to induce DHF. In addition to these hypotheses, mutation was also believed to play a role in DHF[75].

7. Entomology of Vector

Arboviruses are transmitted by hematophagus arthropods which act as vectors. *Ae. aegypti* is the natural vector of dengue virus(es) transmitting the virus from man circulating the virus, directly to other susceptible humans after a period of 8 to 10 days extrinsic incubation. The vector remains active generally under the hot and humid climate and breeds close to human inhabitations. *Ae. aegypti* surveys were undertaken in several parts of the country[18,61,43,76–80] specially during the outbreaks. The surveys revealed that the vector has a fairly wide distribution in India. The mosquitoes were found in high densities in coastal areas along Gangetic and Bramhaputra basins[8,81]. Some cities in Rajasthan and Madhya Pradesh exhibited relatively higher vector densities between July through October (wet season) as compared to February to April (dry season)[78]. In contrast, the vector density enhanced during dry season in Pune[83]. Studies on epidemics in India imply that the onset of an epidemic parallels the build up of mosquito population density[62,78].

Suspected vectors

Aedes albopictus has been considered as a viable vector of dengue viruses in India[18]. However its role in disease transmission is still open to question. While *Ae. aegypti*, is a native of Africa, *Ae. albopictus* is Asian in origin[83]. The latter's involvement in the epidemiology of dengue fever was conjectured even earlier to *Ae. aegypti*[84]. Ecologically too the two species differ. *Ae. aegypti* is predominantly urban and inhabits areas congested with human population, whereas *Ae. albopictus* thrives

luxuriantly in semiurban, rural and sylvan ecosystem. Studies in Malaysia on simians conclusively prove that *Ae. albopictus* alternates the viral cycle via a vertebrate host, other than the man, in a sylvan setup[85]. One strain of DEN 2 virus was isolated in Singapore from *Ae. albopictus* with its infectivity rate determined to 0.8 per cent compared to 18.6 per cent of *Ae. aegypti*[86]. Since identical ecological setup exists in India, the feasibility of *Ae. albopictus* being a potential vector of dengue virus(es) cannot be ruled out. Studies on this mosquito in South East Asia reveal that *Ae. albopictus* is by and large associated with such dengue infection which induce only benign clinical symptoms.

One strain of DEN 3 was also isolated from another mosquito *Culex tritaeniorhyncus* in Phillipines[44]. Being a vector of Japanese encephalitis virus in India, the role of this vector in the epidemiology of dengue viruses can hardly be visualized.

8. Conclusion

Infections caused by dengue serotypes are widely prevalent in India since 1956 and reported to recur almost annually. It may be observed that DEN 2 virus which initially caused only sporadic outbreaks with benign clinical symptoms in Vellore, abruptly induced severe epidemic in Calcutta in 1963 during the first phase. In general benign form of dengue viruses predominated the epidemics in India, though severe forms were also in evidence at some places. Accumulated evidences over the years in South East Asia tend to indicate that DEN 2 and possibly DEN 1 viruses induced severe syndromes (DHF/DSS) in these countries[49,87]. In India too DEN 2 virus has been largely associated in the epidemics manifesting DHF at some places either alone or in combination with other serotypes or Group A Chikungunya virus.

India is endemic to dengue infections as established by serological surveys in various parts of the country. However, the survey is seemingly inadequate in northern, central and north eastern sectors of the country. Since children are the vulnerable target of the severity of disease, it would be pertinent to survey them for immunity levels, periodically. Exploration of comparatively newer serological techniques like immunofluroscence, immunodiffusion (to detect mixed antigen-antibody reactions)[88] and Enzyme linked Immunosorbent Assay (ELISA) and Immunoperoxidase (to detect and assay both, the aetiological agent and the specific antibody produced against it), is suggested. Clinical symptoms are indicator of a possible aetiological agent and may therefore help in the expeditious serodiagnosis[89].

Role of *Ae. albopictus* as the potential vector of dengue virus(es) has been partially established in South East Asia. However its contribution or a definite role in the epidemiology of dengue infections in India, is yet to be confirmed.

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References

1. Kennedy, R. S., *Indian Med. Gaz.*, **47** (1912), 436.
2. Solemankhan, *Indian Med. Gaz.*, **48** (1913), 206.
3. Dacosta, A. S. W., *Indian Med. Gaz.*, **59** (1924), 450.
4. Karamchandani, P. V., *Indian Med. Gaz.*, **72** (1937), 532.
5. Knowles, R. & Das Gupta, B. M., *Indian Med. Gaz.*, **72** (1924), 26.
6. Sabin, A. B., *J. Immunol.*, **1** (1952), 30.
7. Ramachandra Rao, T., *Indian J. Med. Res.*, **63** (1975), 1219.
8. Ramachandra Rao, T., *Indian J. Med. Res.*, **52** (1964), 719.
9. Ramachandra Rao, T., *Bull. W.H.O.*, **44** (1971), 585.
10. Halstead, S. B., Thirayodhin, P. & Olsson, R. A., *Bull. W.H.O.*, **35** (1966), 56.
11. Carey, D. E., Myres, R. M., Reuben, R. & Rodrigues, F. M., *Am. J. Trop. Med. Hyg.*, **15** (1966), 580.
12. Myres, R. M., Carey, D. E., Rodrigues, F. M. & Klantz, C. E., *Indian J. Med. Res.*, **52** (1964), 559.
13. Sarkar, J. K., Pavri, K. M., Chatterjee, S. N., Chakravarty. S. K. & Anderson, C. R., *Indian J. Med. Res.*, **52** (1964), 684.
14. Pavri, K. M., Banerjee, G., Anderson, C. R. & Aikat, B. K., *Indian J. Med. Res.*, **52** (1964), 692.
15. Aikat, B. K., Konar, N. R. & Banerjee, G., *Indian J. Med. Res.*, **52** (1964), 660.
16. Ramakrishnan, S. P., Gelfand, G. M., Bose, P. N., Sehgal, P. N. & Mukherjee, R. N., *Indian J. Med. Res.*, **52** (1964), 633.
17. Sarkar, J. K., Chatterjee, S. N. & Chakravarty, S K., *Indian J. Med. Res.*, **52** (1964), 651.
18. Ramachandra Rao, T. & Anderson, C. R., *Indian J. Med. Res.*, **52** (1964), 727.
19. Paul, S. D., Dandawate, C. N., Banerjee, K. & Krishnamurthy, K., *Indian J. Med. Res.*, **53** (1965), 777.
20. Krishnamurthy, K., Kasturi, T. E. & Chittipantul, U. G., *Indian J. Med. Res.*, **53** (1965), 800.
21. Carey, D. E., Myers, R. M., De Ranitz, C. M., Jadhav, M. & Reuben, R., *Trans. R. Soc. Trop. Med. Hyg.*, **63** (1969), 434.
22. De Ranitz, C. M., Myers, R. M., Varkey, M. J., Issac, Z. H. & Carey, D. E., *Indian J. Med. Res.*, **53** (1965), 756.
23. Rodrigues, F. M., Patankar, H. R., Banerjee, K., Bhatt, P. N., Goverdhan, M. K., Pavri. K. M. & Vittal, M., *Bull. W.H.O.*, **46** (1972), 173.
24. Myers, R. M., Carey, D. E., Banerjee, K., Reuben, R. & Ramamurti, D. V., *Indian J. Med. Res.*, **56** (1968), 781.
25. Sehgal, P. N., Kalra, N. L., Pattanayak, S., Wattal, B. L. & Srivastava, J. B., *Bull. Ind. Soc. Com. Dis.*, **4** (1967), 91.
26. Rodrigues, F. M., Pavri, K. M., Dandawate, C. N., Banerjee, K. & Bhatt, P. N., *Indian. J. Med. Res.*, **61** (1973), 1462.
27. Myers, R. M., Carey, D. E., De Ranitz, C. M., Reuben, R. & Bannet, B., *Indian J. Med. Res.*, **57** (1969), 1392.
28. Sarkar, J. R., Ghosh. J. M., Chatterjee, S. N. & Chakravarty, S. K., *Indian J. Med. Res.*, **58** (1970), 151.
29. Balaya, S., Paul S. D., D'lima, L. V. & Pavri, K. M., *Indian J. Med. Res.*, **57** (1969), 767.
30. Myers, R. M., Varkey, M. J., Reuben, R. & Jessudass, E. S., *Indian J. Med. Res.*, **58** (1970), 24.
31. Chaturvedi, U. C., Kapoor, A. K., Mathur, A., Chandra, D., Khan, A. M. & Mehrotra, R. M. L., *Bull. W.H.O.*, **43** (1970), 281.
32. Chaturvedi, U. C., Mathur, A., Kapoor, A. K., Mehrotra, N. K. & Mehrotra, R. M. L., *Bull. W.H.O.*, **43** (1970b), 289.
33. Chaturvedi, U. C., Mathur, A., Kapoor, A. K., Tandon, H. O. & Mehrotra, R. M. L., *Indian J. Med. Res.*, **60** (1972), 329.
34. Ghosh, S. N., Pavri, K. M., Singh, K. R. P., Sheikh, B. H., D'lima, L. V., Mahadev, P. V. & Ramachandra Rao, T., *Indian J. Med. Res.*, **62** (1974), 511.
35. Arora, D. D., Pattanayak, S. & Raghavan, N. G. S., 'Annual Report' (National Instt. of Communicable Diseases, New Delhi), 1970, p. 31.
36. George, S. & Soman, R. S., *Indian J. Med. Res.*, **63** (1975), 396.

37. Raghavan, N. G. S., Pattanayak, S., Arora, D. D., Singha, P., Mathur, P. S. & Ghosh, T. K., 'Annual Report' (National Instt. of Communicable Diseases, New Delhi), 1970, p 32.
38. Chaturvedi, U. C., Mathur, A., Kapoor, A. K., Agarwal, S. K., Tandon, H. O. & Mehrotra, R. M. L., *Indian J. Med. Res.*, **62** (1974), 627.
39. Padbidri, V. S., Dandawate, C. N., Goverdhan, M. K., Bhat, U. K. M., Rodrigues, F. M., D'lima, L. V., Kaul, H. N., Guru, P. Y., Sharma, R. & Gupta, N. P., *Indian J. Med. Res.*, **61** (1973), 1737.
40. Mathew, T., Suri, N. K., Bhola, S. R., Suri, J. C., Arora, R. R. & Lahiri, S. K., *Indian J. Med. Res.*, **64** (1976), 1136.
41. Mathew, T., Suri, J. S., Suri, N. K., Bhola, S. R., Arora, R. R., Lal, P., Raichoudhary, A. N., Mathur, K. K. & Gupta, J. P., *Indian J. Med. Res.*, **65** (1977), 613.
42. Sreenivasan, M. A., Rodrigues, F. M., Venkateshan, C. N. & Jaya Ram Panickar, C. K., *Indian J. Med. Res.*, **69** (1979), 538.
43. Soman, R. S., *Indian J. Med. Res.*, **65** (1977), 8.
44. Hammon, W. McD., Rudnick, A. & Sather, G. E., *Science*, **131** (1960), 1102.
45. Hammon, W. McD. & Sather, G. E., *Am. J. Trop. Med. Hyg.*, **13** (1964), 629.
46. Carey, D. E., Rodrigues, F. M., Myers, R. M. & Webb, J. K. G., *Indian Ped.*, **5** (1968), 285.
47. Lim, K. A,, Chen, Y. C., Phoon, W. O. & Hanam, E., *Bull. W.H.O.*, **30** (1964), 227.
48. Myers, R. M. & Varkey, M. J., *Indian J. Med. Res.*, **58** (1970), 1301.
49. Halstead, S. B., *Bull. W.H.O.*, **35** (1966), 3.
50. Sabin, A. B. & Schlesinger, R. W., *Science*, **101** (1945), 640.
51. Pavri, K. M. & Ghosh, S. N., *Bull. W.H.O.*, **40** (1969), 984.
52. Paul, S. D., Banerjee, K. & D'lima, L. V., *Indian J. Med. Res.*, **53** (1965), 790.
53. Paul, S. D. & Banerjee, K., *Indian J. Med. Res.*, **53** (1965), 405.
54. Singh, K. R. P. & Paul, S. D., *Bull. W.H.O.*, **40** (1969), 982.
55. Russel, P. K. & Brandt, W. E., 'In Perspectives in Virology' Editted by Morris Pollard (Academic Press, New York), 1973, p. 263.
56. Sarkar, J. K., *Bull. W.H.O.*, **35** (1966), 59.
57. Carey, D. E., Myers, R. M. & Reuben, R., *Science*, **143** (1964), 131.
58. Smithburn, K. C., Kerr, J. A. & Gatne, P. B., *J. Immunol*, **72** (1954), 248.
59. Banerjee, K. & Desai, P. K., *Indian J. Med. Res.*, **61** (1973), 344.
60. Sarkar, J. K. & Chatterjee, S. N., *Indian J. Med. Res.*, **50** (1962), 833.
61. Mukherjee, K. K., Chakravarty, S. K., Sarkar, J. K., Chakravarty, M. S., Roy, S., Das, B. C. & Mitra, A. C., *Indian J. Pathol. Microbiol.*, **20** (1977), 169.
62. Rodrigues, F. M. & Dandawate, C. N., *Indian J. Med. Res.*, **64** (1977), 453.
63. Chaturvedi, U. C., Bahuguna, L. N., Mehrotra, N. K., Tandon, H. O. & Mehrotra, R. M. L., *Indian J. Pathol. Microbiol.*, **19** (1976), 161.
64. Sheikh, B. H., Rodrigues, F. M., Padbicri, V. S., Gupta, N. P., Sundram, K. P. & Ghosh, S. N., *Indian J. Med. Res.*, **67** (1978), 175.
65. Halstead, S. B., Nimmannitya, S. & Cohen, S. N., *Yale. J. Biol. Med.*, **42** (1970), 350.
66. Halstead, S. B., Nimmannitya, S., Yamarat, C. & Russal, P. K., *Jap. J. Med. Sci. Biol.*, **20** (1967), 96.
67. W.H.O. Memoranda, *Bull. W.H.O.*, **48** (1973), 117.
68. Winter, P. E., Yuill, T. M., Udomskadi, S., Gould, D., Mantapanich, S. & Russel, P. K., *Am. J. Trop. Med. & Hyg.*, **17** (1968), 590.
69. Scott McNair, R., Nimmannitya, S., Bacraft, W. H. & Manuwan, P., *Am. J. Trop. Med. Hyg.*, **25** (1976), 866, *Virol. Abstr.*, **10V** (1977), 4987.
70. Briody, B. A. & Gills, R. E., 'Ecology of Arboviruses in Microbiology of Infectious Disease' (McGraw Hills, New York), 1974, 563.
71. Bokish, V. A., Top, F. M. (Jr), Russel, P. K., Dixon, F. J. & Muller Eberhard, M. J., *New. Eng. J. Med.*, **289** (1973), 996.
72. Ruangjrachuporn, U., Bounpuoknang, S. & Nimmannitya, S., *Clin. Exp. Immunol.*, **36** (1979), 36.
73. Pavri, K. M., Sheikh, B. H., Ghosh, S. N. & Chandankar, V. P., *Indian J. Med. Res.*, **66** (1977), 537.
74. Pavri, K. M., *Indian J. Med. Res.*, **64** (1976), 713.

75. Halstead, S. B. & Yamarat, C., *Am. J. Publ. Hlth.*, **55** (1965b), 1368.
76. Wattal, B. L., *Indian J. Med. Res.*, **52** (1964), 710.
77. Kalra, N. L., *Bull. Ind. Soc. Mal. Com. Dis.*, **2** (1965), 101.
78. Kalra, N. L. & Wattal, B. L., 'Annual Report' (National Instt. of Communicable Diseases, Delhi), 1970, p. 58.
79. Rahman, S. J. & Sundaram, R. A. M., 'Annual Report' (National Instt. of Communicable Diseases, Delhi), 1967, 25.
80. Reuben, R., *Indian J. Med. Res.*, **58** (1970), 854.
81. Kalra, N. L., Wattal, B. L. & Raghvan, N. G. S., *Bull. Ind. Soc. Mal. Com. Dis.*, **5** (1968), 307.
82. Geeverghese, G., Kaul, H. N. & Vijai Dhanda, *Indian J. Med. Res.*, **63** (1975), 1155.
83. McLean, D. M., 'Text Book of Virology' Editted by Rhodes, A. J. & Van Rooyen, C. E. (The William & Wilkins Co., Baltimore, 1968, p. 717.
84. Simmon, J. S., St. John, J. H. & Reynolds, F. H. K., *Am. J. Trop. Med. & Hyg.*, **10** (1930), 17 (Cited in ref. No 8).
85. Rudnick, A., *Bull. W.H.O.*, **35** (1966), 78.
86. Rudnick, A., *Bull. W.H.O.*, **32** (1960), 63.
87. Hammon, W. McD., *Am. J. Trop. Med. & Hyg.*, **22** (1973), 82.
88. W.H.O. Memoranda, *Bull. W.H.O.*, **35** (1966), 17.
89. Work, T. H. & Pazon, M., 'Human and Related Viruses'. Edited by Kurstak, E. and Kurstak, C. (Academic Press, New York), 1967, p. 621.