Tropical Medicine and International Health

VOLUME 9 NO 4 PP 499–507 APRIL 2004

Dengue vector prevalence and virus infection in a rural area in south India

S. C. Tewari[1], V. Thenmozhi[1], C. R. Katholi[2], R. Manavalan[1], A. Munirathinam[1] and A. Gajanana[3]

1 *Centre for Research in Medical Entomology, Indian Council of Medical Research, Madurai, India*
2 *Department of Biostatistics, University of Alabama at Birmingham, AL, USA*
3 *Retired Officer of Centre for Research in Medical Entomology, Madurai, India*

Summary We conducted a 2-year (1997–1999) longitudinal, entomological and virological study in three dengue endemic villages in Vellore district, Tamil Nadu, to understand the dynamics of dengue transmission. *Aedes aegypti* (Linn.), *Ae. albopictus* (Skuse) and *Ae. vittatus* (Bigot) were the prevalent vector species. *Aedes aegypti* was breeding throughout the year with a Breteau index ranging from 9.05 to 45.49. *Aedes albopictus* and *Ae. vittatus* were prevalent mainly in the rainy season. Small water holding containers (cemented tanks/cisterns) were the perennial breeding source of *Ae. aegypti*, and its abundance was significantly higher in semi-urbanized central areas than the peripheral areas of the villages. From 271 pools (4016 specimens) of adult females, eight dengue virus (DENV) isolates were obtained of which seven were from *Ae. aegypti* and one from *Ae. albopictus*. This is the first report of DENV isolation from *Ae. albopictus* in rural India. Infection rates in the two species were comparable. However, due to higher and perennial prevalence, *Ae. aegypti* is considered as primary vector with *Ae. albopictus* playing a secondary role. Despite circulation of all four serotypes (DENV 1–4) detected mainly during the transmission season, the high anthropophilic index of the vectors and their abundance, no human dengue case was reported, suggesting silent dengue transmission.

keywords dengue virus isolation, silent dengue transmission, vector infection, Breteau index, *Aedes aegypti*, *Aedes albopictus*

Introduction

Dengue is currently one of the most important arboviral diseases, with 2.5 billion people living in areas of risk and many tens of millions of cases occurring each year (Halstead 1980; Gubler 1998). It is one of the most rapidly rising mosquito transmitted infections in the world (Lam 1993) and has been identified as a re-emerging disease in southeast Asia (WHO 1999). Dengue has been known in India since 1945 (Sabin 1952), and the classical dengue fever (DF) was mainly associated with febrile illness and joint pains. The severe form of infection manifests as dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS); 44% of these cases can be fatal (Rigau-Perez *et al.* 1998). In India, the first outbreak of DHF/DSS was documented in Delhi in 1988 (Kabra *et al.* 1992). But prior to this, endemic transmission of all four dengue serotypes had been reported (WHO 1997).

In southern India, dengue was mainly an urban disease in the 1960s and 1970s, associated with the container breeding vector *Aedes aegypti*. Many isolations of all the four serotypes of dengue virus (DENV) were made from pools of *Ae. aegypti*; for the first time DEN1 and DEN4 (five isolates) in 1961 (Carey *et al.* 1964), DEN2 (two isolates) in 1966 (Myers *et al.* 1969) and 36 isolates of all the four serotypes in 1968 (Myers *et al.* 1970). During that time there was little storage of water in villages and therefore *Aedes* species were scarce (Reuben 1971a,b) and dengue was absent. Subsequently, with the introduction of piped water supply, dengue made incursions to rural areas of south India and outbreaks have been reported (Abdul Kader *et al.* 1997; Singh *et al.* 2000). In rural areas of India, the role of *Ae. aegypti* as a principal vector had already been well documented (Ilkal *et al.* 1991; Mahadev *et al.* 1993) but the role of *Ae. albopictus* was not defined. Although *Ae. albopictus* has been considered a potential vector of dengue and several virus isolations have been made in southeast Asia (Hawley 1988), in India isolation of DENV had been documented only once in the east (Reuben *et al.* 1988).

In 1990, an insular outbreak of DF was reported in one village near Vellore town (Norman *et al.* 1991) followed by an epidemic of DHF/DSS in and around Vellore district (Cherian *et al.* 1994), but no virus isolation was attempted during these outbreaks. Since the epidemiology of rural dengue is poorly understood, a prospective study was

Tropical Medicine and International Health VOLUME 9 NO 4 PP 499–507 APRIL 2004

undertaken in three endemic villages in Vellore district to comprehend the dynamics of dengue transmission with particular reference to breeding habitats, abundance and virus infection rates in different vector species.

Materials and methods

Study sites

Three villages, Munjurpattu (where an isolated dengue outbreak occurred in 1990), Kaniambadi and Pennathur, were selected for the study. They are situated in North Arcot district in south India. The villages have a population of 9476 and 2055 houses and are well connected by road to Vellore town 3–5 km away. Each village has two well-demarcated localities, a semi-urbanized central part with residents from higher, relatively better-off socioeconomic strata and a peripheral colony where the residents are mainly craftsmen and labourers. The area receives some rainfall from the end of May (southwest monsoon), and heavy rains from September to December under the influence of northeast monsoon.

Entomological study

Fourteen surveys were carried out between March 1997 and February 1999 on average 40 days apart. A demographic map of the study villages was prepared and houses to be examined in each survey were marked.

Larval survey

In each survey, about 25 % of houses in each village were searched both inside and outside for breeding places of *Aedes* using single larval technique (Sheppard *et al.* 1969). From March 1997 to December 1997, each larva was individually reared and identified at the adult stage. As only three species of *Aedes* (*Ae. aegypti, Ae. albopictus* and *Ae. vittatus*) were found, from January 1998 the identification was carried out at the fourth instar stage. Breteau index (BI), house index (HI) and container index (CI) were calculated (WHO 1999).

Adult survey

Mosquitoes resting or flying inside the houses were collected in the morning for 15 min per house using a mouth aspirator and flash light. In each village, per survey, two insect collectors spent 2 h each (four man hours per village), and the average number of adults per man hour (PMH) was estimated. *Aedes* species landing on human volunteers (from whom informed consent was obtained) were collected in the morning and late afternoon for 30 min per volunteer, and density was expressed as females landing PMH. To enhance the sample size for virus isolation, additional collections were made from the localities where higher density was observed. A sample of blood engorged females was used for host blood meal identification by gel diffusion test (Reuben *et al.* 1992). The remaining specimens were held in the field laboratory for 24–48 h for digestion of their blood meal, subsequently pooled (pool size ranged from 1 to 28 females), stored in liquid nitrogen and transferred to the laboratory in Madurai for virus isolation.

Dengue virus isolation study

Antigen capture enzyme linked immunosorbent assay (ELISA)

Female mosquitoes collected from June 1997 to February 1999 were tested for detection of flavivirus by ELISA (Gajanana *et al.* 1995a). Each pool was triturated in 0.6 ml of 0.75% bovine albumin phosphate buffered saline with pH 7.4 and the suspension centrifuged at RCF 6000 *g* at 4 °C for 30 min and further screened using the capture monoclonal antibody (MAb), D14G2 (1:1000) and the detector MAb 6B6C-1 tagged to horse-radish peroxidase (1:2000). As both the MAbs were broadly reactive against flaviviruses, pools with optical density (OD) ≥ mean + 4 SD of the OD of the laboratory colonized *Ae. aegypti* pools were considered positive for flaviviruses.

Insect-bioassay (TOXO-IFA) (Gajanana *et al.* 1995a). Aliquots of each flavivirus positive pool were inoculated intracerebrally to 50 early third instar *Toxorhynchites splendens* larvae and incubated for 14 days at 32 °C. Head squash preparations were examined by indirect immunofluroscent assay (IFA) using dengue virus-specific MAbs. For screening, six inoculated larvae were examined using dengue broad reacting MAb D2-9D12-5-24 and flouresc-ein isothiocyanate conjugated anti-mouse immunoglobulin (Dakoppats, Denmark). A pool was considered positive for DENV if at least one of six larvae was positive by IFA. Subsequently, the rest of the larvae initially inoculated by the same positive pool were tested using DEN 1–4 type specific MAbs [DEN1(D2-IF1-3), DEN2(3H5-1-21), DEN3(D6-8A1-12), DEN4(IH10-6-7)] provided by Dr D.J. Gubler and Dr T.F. Tsai, CDC, Fort Collins, USA. For each serotype MAb, at least six larvae were used.

Statistical analysis

Analyses of entomological parameters were carried out using SPSS 11.5. Due to low adult density, the pool size of

Tropical Medicine and International Health VOLUME 9 NO 4 PP 499–507 APRIL 2004

adult females for virus isolations varied between 1 and 28 specimens per pool. The minimum infection rate (MIR) is not an appropriate parameter for expressing infection rates and for comparison between vector species when pool sizes are unequal. To overcome this problem POOL SCREEN II software was used which extends the methods described for equal pools (Katholi *et al.* 1995). This takes into account results of negative as well as positive tests. Virus infection rate (based on ELISA/IFA results) was expressed as maximum likelihood estimate with 95% confidence intervals.

Results

Entomological survey

In each survey, an average of 522 houses and 1397 water containers lying in and around the human dwellings were examined for immature stages of *Aedes* species. About 25% of houses and 15% of containers were found positive (Table 1).

The primary vector, *Ae. aegypti*, was found to be well established in the rural areas as it was found breeding throughout the year. The average *Stegomyia* indices were HI 16.42, CI 8.81 and BI 24.77 (Table 1). Its density was lowest during May 1998 (hot season) when HI, CI and BI were 7.04, 4.02 and 9.05, respectively, whereas highest in August and October 1998 (rainy season) when the density increased to 26.2, 13.77 and 45.49, respectively. The secondary vector, *Ae. albopictus*, was highly seasonal. Its density decreased almost to zero level during the hot season (March–June) but sharply increased in the rainy seasons with peak abundance (BI 40.5) during November 1997. The larval abundance of both the species was positively correlated ($r^2 = 0.3636$ for *Ae. aegypti* and 0.2089 for *Ae. albopictus*) with rainfall (Figure 1). *Aedes vittatus* followed a similar trend ($r^2 = 0.5933$) of seasonality as in the case of *Ae. albopictus* (Table 1).

Mainly five types of water holding containers were found infested with the *Aedes*. These were: (i) cement tank (CT), fixed concrete triangular/rectangular tank, fabricated at ground level with a capacity of about 100–200 l; (ii) cement cistern (CC), portable, thin walled cemented cylindrical container of 20–50 l capacity; (iii) mud pot (MP), traditional earthen pot made locally with a capacity of 5–20 l; (iv) metal and plastic containers (MPC), used for storing potable water and (v) discarded containers (DC), which included unused grinding stones, used tyres, broken mud pots, discarded plastic/metal containers, animal feeding trough, etc., which were found in and around the houses as waste materials. Among the positive containers [$n = 19\,559$; range: 8.41% (CC) to 25.25% (DC)], *Ae. aegypti* contributed about 56% followed by *Ae. vittatus* (24%) and *Ae. albopictus* (20%). Cement tanks were

Table 1 Larval and adult abundance of three *Aedes* species prevalent in index villages

	Larval abundance (three species of *Aedes* combined)		*Ae. aegypti*					*Ae. albopictus*				*Ae. vittatus*		
			Larval indices			Adult female abundance (PMH)		Larval indices			Adult female abundance (PMH)	Larval indices		
Months	% Houses positive (no. examined)	% Containers positive (no. examined)	HI*	CI*	BI*	Resting	Landing	HI	CI	BI	Landing	HI	CI	BI
Mar-97	14.61 (486)	7.23 (1341)	13.17	6.68	18.52	6.00	17.46	0.82	0.30	0.82	0.00	0.62	0.22	0.62
Apr-97	19.44 (468)	10.29 (1312)	17.95	9.57	26.92	28.00	9.37	0.00	0.00	0.00	0.00	1.71	0.61	1.71
Jun-97	15.63 (352)	7.96 (955)	12.22	5.52	17.33	6.38	11.74	0.00	0.00	0.00	0.00	3.98	1.36	4.26
Aug-97	29.07 (540)	17.07 (1470)	17.78	9.13	25.00	17.67	12.00	0.74	0.54	1.48	0.00	14.44	7.30	20.00
Sep-97	38.28 (512)	27.43 (1345)	22.66	13.04	34.38	21.11	32.00	10.74	5.26	13.87	5.14	17.77	9.04	23.83
Nov-97	45.25 (484)	29.78 (1484)	24.59	12.53	38.43	39.26	18.00	25.00	13.21	40.50	22.13	10.12	4.04	12.40
Jan-98	22.85 (477)	14.77 (1097)	16.98	9.85	22.64	12.60	14.93	5.45	2.73	6.29	12.53	4.82	2.19	5.03
Mar-98	10.24 (654)	7.22 (1495)	9.93	6.97	15.90	11.38	40.00	0.31	0.13	0.31	0.00	0.46	0.20	0.46
May-98	7.47 (696)	4.40 (1567)	7.04	4.02	9.05	9.36	12.24	0.43	0.26	0.43	0.00	0.29	0.13	0.29
Jun-98	15.11 (655)	8.29 (1593)	11.91	6.03	14.66	6.38	18.50	0.15	0.06	0.15	0.00	4.12	2.20	5.34
Aug-98	45.69 (499)	27.56 (1651)	23.85	13.73	45.49	16.80	6.50	9.22	3.57	9.22	2.69	25.25	10.22	33.87
Oct-98	45.20 (500)	28.35 (1633)	26.20	13.53	44.20	10.42	14.17	15.60	7.10	15.60	8.57	20.60	8.33	27.20
Dec-98	31.01 (474)	17.20 (1407)	14.56	6.97	20.68	8.13	4.89	14.35	6.75	14.35	3.33	8.02	3.48	10.34
Feb-99	13.04 (506)	7.11 (1209)	11.07	5.70	13.64	4.24	6.14	1.78	0.74	1.78	0.50	0.79	0.41	0.99
Mean	25.21 (522)	15.33 (1397)	16.42	8.81	24.77	14.12	15.57	6.04	2.90	7.49	3.92	8.07	3.55	10.45
SD	13.79 (90.28)	9.32 (204.55)	6.02	3.31	11.63	9.84	9.80	7.83	3.94	11.19	6.50	8.33	3.65	11.31

Tropical Medicine and International Health VOLUME 9 NO 4 PP 499–507 APRIL 2004

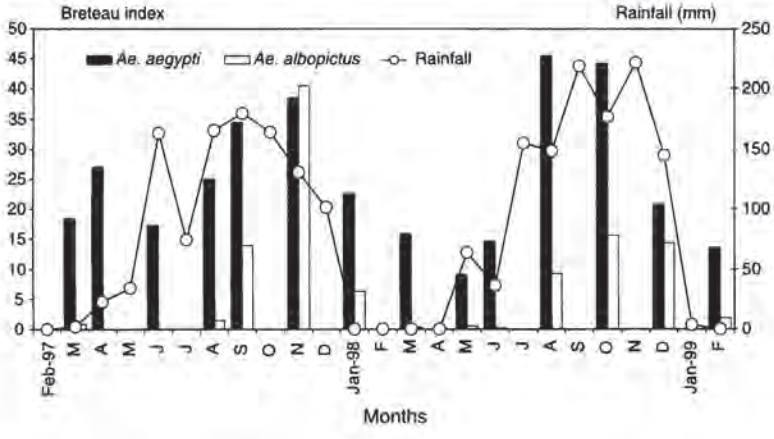

Figure 1 Comparison of larval densities of dengue vectors with rainfall.

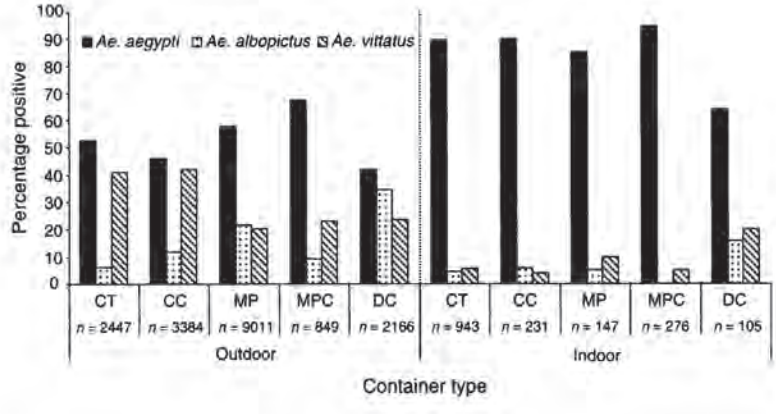

Figure 2 Breeding habitats of *Aedes* species.

preferred by *Ae. aegypti* and MP and DC by *Ae. albopictus*. *Aedes vittatus* was seen almost in equal proportions in all the five types of containers but showed a slightly higher preference (34%) to CC (Figure 2). The number of containers lying outside (OD) in peridomestic areas was about 10 times more than those found indoors (ID). *Aedes aegypti* preferred ID containers whereas *Ae. albopictus* and *Ae. vittatus* preferred OD (Figure 2). Cement tanks were a perennial source of breeding, while other habitats were unproductive in hot months (Figure 3).

Comparing the prevalence of the three *Aedes* species in the central and peripheral localities of the villages (Figure 4), the BI of *Ae. aegypti* in the central locality (GM ± SE: 32.23 ± 1.27) was significantly higher than that of peripheral colony (9.12 ± 1.39) ($t = 12.63$, d.f. = 26, $P < 0.001$). But with *Ae. albopictus*, the BI values of central locality (4.59 ± 2.11) and in the periphery (3.87 ± 1.96) were comparable ($t = 1.86$, d.f. = 26, $P > 0.05$). Similar values were obtained with *Ae. vittatus* also: in the central locality (6.98 ± 1.86) and in the periphery (5.6 ± 1.92, $t = 1.96$, d.f. = 26, $P > 0.05$).

The abundance of adult *Ae. aegypti* fluctuated very much, without correlation between PMH densities in resting and landing collections ($r^2 = 0.016$). The larval density positively correlated with adult resting ($r^2 = 0.325$) but not with the landing density ($r^2 = 0.002$). *Aedes aegypti* was biting humans and resting inside the houses year round; the landing rate ranged from 4.9 to 40.0 (mean 15.57) and resting density ranged from 4.24 to 39.26 (mean 14.12) (Table 1).

Aedes albopictus was breeding only during the rainy season and the larval and adult densities were positively correlated ($r^2 = 0.793$). Adult females were collected only in landing collections biting humans outdoors, around the houses and preferably near vegetation. No specimen was captured in indoor resting collections. This species was

Tropical Medicine and International Health VOLUME 9 NO 4 PP 499–507 APRIL 2004

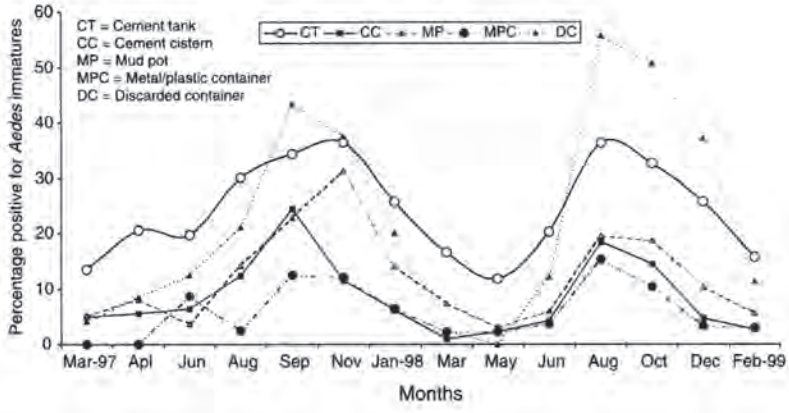

Figure 3 Breeding of *Aedes* species in different types of habitats.

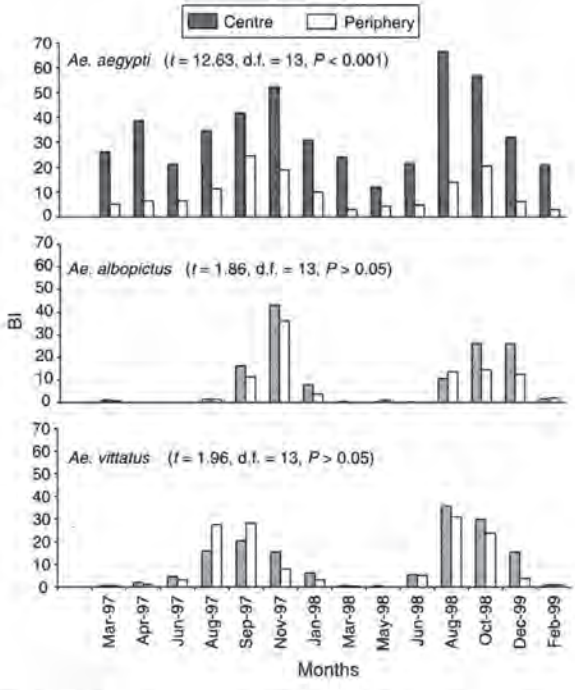

Figure 4 Comparison of larval density of three *Aedes* species between periphery and centre of village.

recorded only in seven occasions (wet rainy season) with PMH density ranging between 0.5 (February) and 22.1 (November) (Table 1). *Aedes vittatus* was not found in adult collections, except on one occasion when 18 specimens were captured after 14 man-hours in landing collection during rainy season.

All samples of blood meals of both species were tested against antisera to humans, cattle, pigs, horses, dogs and fowl. Host feeding analysis showed that both *Ae. aegypti* and *Ae. albopictus* were highly anthropophilic as 90.04% (434/482) and 96.4% (27/28), respectively, had fed on humans. Cattle feeds accounted for 1.45%, goat and fowl feeds were extremely low (0.21% each) in the case of *Ae. aegypti*. There was 9.54% and 3.57% of blood meal were found negative against all the antisera tested for *Ae. aegypti* and *Ae. Albopictus*, respectively.

Dengue virus isolations

Altogether 236 pools (3640 specimens) of *Ae. aegypti*, 33 pools (363 specimens) of *Ae. albopictus* and two pools (13 specimens) of *Ae. vittatus* were tested by ELISA. Of these, 10 pools of *Ae. aegypti* and two of *Ae. albopictus* were positive for flavivirus infection (Table 2). No infection was recorded in *Ae. vittatus*. Of the 12 flavivirus positive pools, eight were identified as DENV using dengue-specific, broad-reactive MAb by the Toxo-IFA system. Seven isolates were from *Ae. aegypti* and one from *Ae. albopictus*. Further tests using serotype-specific MAb showed that four isolates were DEN2, one isolate each was DEN1, DEN3 and DEN4, and one was mixed DEN3 and DEN4. Of these, five (62.5%) were detected during rainy seasons (September–December), which is the DENV transmission season in this area. Maximum isolates were recorded (four in number) from Munjurpattu where an epidemic occurred in 1990 (Norman *et al.* 1991).

Maximum likelihood estimates of flavivirus (ELISA) and DENV (IFA) infections for the total 271 pools (three species combined) were 0.00305 and 0.00202, respectively. In both the tests, *Ae. albopictus* showed a higher rate of infection (ELISA, 0.00559; IFA, 0.00278) than *Ae. aegypti* (ELISA, 0.00281; IFA, 0.00195). However, 95% confidence intervals of the two species overlap, and hence the difference is not significant (Table 3). During the whole

Tropical Medicine and International Health VOLUME 9 NO 4 PP 499–507 APRIL 2004

Table 2 Dengue virus infection in *Aedes aegypti* and *Aedes albopictus*

	Pools examined by ELISA for flavivirus (no. of females)		Pools positive for flavivirus		Dengue virus serotype determined by TOXO-IFA	
Months	*Ae. aegypti*	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*	*Ae. aegypti*	*Ae. albopictus*
Jun-97	6 (77)	0	0	0	–	–
Aug-97	16 (203)	0	0	0	–	–
Sep-97	23 (341)	3 (25)	1	0	DEN 3+4	–
Nov-97	27 (407)	4 (39)	0	0	–	–
Jan-98	29 (517)	3 (27)	1	0	DEN 2	–
Mar-98	19 (282)	0	0	0	–	–
May-98	21 (339)	0	0	0	–	–
Jun-98	15 (203)	0	1	0	–	–
Aug-98	27 (477)	4 (54)	1	1	DEN 2	–
Oct-98	16 (245)	9 (148)	4	0	DEN 3	–
				0	DEN 4	
Dec-98	21 (338)	7 (62)	1	1	DEN 2	DEN 2
Feb-99	16 (211)	2 (7)	1	0	DEN 1	–
Total	236 (3640)	33 (363)	10	2	7	1

Table 3 Maximum likelihood estimate of dengue virus infection in three *Aedes* species using POOL SCREEN software

	Mosquito pools positive by ELISA/examined (no. of female specimens)			
Parameters	All the three species of *Aedes* (combined)	*Ae. aegypti*	*Ae. albopictus*	*Ae. vittatus*
	12/271 (4016)	10/236 (3640)	2/33 (363)	0/2 (13)
Flavivirus infection (ELISA)				
Point estimator				
Maximum likelihood estimate	0.00305	0.00281	0.00559	–
95% Confidence intervals				
Clopper–Pearson fiducial	0.00158, 0.00535	0.00135, 0.00517	0.00068, 0.02058	–
Dengue virus infection (IFA)				
	8/271	7/236	1/33	0/2
Point estimator				
Maximum likelihood estimate	0.00202	0.00195	0.00278	–
95% Confidence intervals				
Clopper–Pearson fiducial	0.00088, 0.00399	0.00079, 0.00403	0.00007, 0.01556	–

study period no apparent dengue case was reported nor was there any unusual increase in febrile cases in humans.

Discussion

For the entomological surveillance of dengue vectors, traditional *Stegomyia* indices (HI, CI and BI) were used as per WHO guidelines (WHO 1999). Focks and Chadee (1997) and Focks *et al.* (2000) have suggested replacement of these traditional indices by pupal survey, but the method is labour intensive for routine application (Gubler 1997). Among the indices, as BI is simple to adopt and logistically better than CI and HI (Reiter & Gubler 1997), we used BI for comparative analysis.

In the 1990 epidemic of DF it was mainly mudpots used as water storage containers that supported *Ae. aegypti* breeding (Norman *et al.* 1991). But we found *Ae. aegypti* breeding mainly in small cement tanks and cisterns. The cement tanks could not be drained out completely due to lack of proper outlets and therefore retained small quantities of water sufficient for immature stages to thrive. Thus, this habitat remained a perennial source of breeding throughout the year. In Maharashtra (Ilkal *et al.* 1991), Samurai Island (Thavara *et al.* 2001) and Domican

Tropical Medicine and International Health VOLUME 9 NO 4 PP 499–507 APRIL 2004

Republic (Tidwell *et al.* 1990), infestation of *Ae. aegypti* was also mainly in cement containers. However, in western India (Rajasthan), breeding was mainly in mudpots (Reuben & Panickar 1975).

Among the three *Aedes* species recorded, *Ae. aegypti* was always predominant in all the habitats (Figure 2). This species showed a distinct preference to the semi-urbanized central part of the village whereas *Ae. albopictus* and *Ae. vittatus* showed no such preferences (Figure 4). This suggests that partial urbanization led to establishment of *Ae. aegypti* in villages. This mosquito was found biting humans throughout the year with 90% average anthropophilic index confirming its well-known anthropophilic nature (Rodhain & Rosen 1997). We could isolate all the four serotypes from mosquito samples collected in villages around Vellore town indicating that the serotypes which were demonstrated during the 1960s (Myers *et al.* 1970) are well established in this area. More than 85% of the total confirmed DENV isolates were from *Ae. aegypti*, indicating that this species was the primary vector of dengue in villages in Vellore. The infection rate appears to be lower than reported by others. During epidemics in Maharashtra, three out of 375 (Ilkal *et al.* 1991), in Gujarat, two out of 225 (Mahadev *et al.* 1993) and in Ahmedabad, seven out of 36 (Joshi *et al.* 2000) specimens examined were found infected. In Singapore, the average MIRs were 57.6 (Chow *et al.* 1998). In a silent dengue situation in Senegal (Diallo *et al.* 2003), the infection rate was 2.74. However, it is not appropriate to make a direct comparison because of different methodologies used in different studies.

Aedes albopictus was prevalent mainly during rainy seasons preferring to breed outdoors in discarded containers, an observation similar to that made by Hawley (1988). In our study, there was only one isolation of DENV from *Ae. albopictus* – the first record of DENV isolation from field-collected females in rural India. The infection rate and anthropophilic index were comparable to those of *Ae. aegypti*. However, because of its low density compared with *Ae. aegypti* and its seasonal nature of prevalence it could be possibly acting as a secondary vector.

Though *Ae. vittatus* was found breeding throughout the year and mean larval indices were higher than those of *Ae. albopictus* (Table 1), very few adults were captured in human landing catches. Because of its poor anthropophilic nature and no isolation of virus from a very few adult specimens obtained in the field, *Ae. vittatus* appears to be playing no role in dengue transmission in the study villages.

During the study period there was circulation of all the four dengue virus serotypes (hyper-endemic), a high anthropophilic index of the vectors and vector abundance throughout the year – a setting ideal for human dengue transmission. But no apparent clinical case of DF/DHF was reported. Silent dengue infection is now a well-documented phenomenon (Gubler *et al.* 1978; Halstead 1994; Gajanana *et al.* 1995b; Chen *et al.* 1996; Endy *et al.* 2002; Rodrigues *et al.* 2002; Teixeira *et al.* 2002). There is a strong probability of inapparent dengue virus infections causing mild DF in our study population, which would have been missed in the surveillance system and remained unnoticed. This could have been confirmed by a simultaneous serological survey that was not included in our study. However, in a neighbouring district 7–22% dengue seroconversion in rural children (5–12 years) in two consecutive years (1991–1993) was reported by Vijayarani and Gajanana (2000).

Earlier studies on silent dengue are based on serological tests such as enzyme immunoassays and haemagglutination inhibition test. Since DENV serotypes are serologically cross-reactive among themselves and with other flaviviruses, it is often difficult to precisely establish the identity of the virus causing seroconversions especially in an area like Vellore, endemic for more than one flavivirus infection. Therefore, in our unique long-term prospective study, we monitored DENV infection by virus isolations to get an insight into the dynamics of virus circulation. In Senegal, a sylvatic cycle of DENV transmission in mosquitoes was observed during interepidemic periods (Diallo *et al.* 2003). Chow *et al.* (1998) detected infection in *Ae. aegypti* at least 6 weeks and in *Ae. albopictus* 4 weeks prior to epidemic in Singapore. We have isolated DENV strains mainly during the transmission wet months. However, the epidemic of 1990 in the Vellore village occurred in May (a dry season) following unusually heavy rains (Norman *et al.* 1991). Earlier in this area, Thenmozhi *et al.* (2000) have recorded vertical transmission of dengue virus in *Ae. aegypti* during the period when dengue was not traditionally apparent, suggesting that endemicity of virus is maintained by vertical transmission. This shows that a mosquito cycle occurs for perpetuation of the virus in nature and under favourable conditions epidemic may break out any time.

In conclusion, our study shows that villages in southern India are hyper-endemic for dengue with multiple serotypes circulating and vulnerable for outbreaks as recently reported by Victor *et al.* (2002). However, more studies are needed in order to define critical levels of DENV infection in the vectors and vector density to develop early warning systems.

Acknowledgements

We are grateful to Dr K. Satyanarayana, Officer on Special Duty (Director), for his constant persuasion, abundant encouragement and useful suggestions for the preparation

of the paper. We are indebted to Dr Rachel Reuben, Former Director, CRME, for her guidance in the analysis of data and for critically reviewing the manuscript. Assistance rendered in the field work by Dr R. Rajendran, Officer-in-charge of CRME Field Station, Vridhachalam, and all technical staff is gratefully acknowledged. Thanks are due to Shriyuts K. Pazhaninathan and V. Rajamannar for helping in data analysis, and A. Venkatesh for desktop editing of the manuscript and infrastructural facilities provided by the Indian Council of Medical Research, New Delhi.

References

Abdul Kader MS, Paul Kandaswamy, Appavoo NC & Anuradha L (1997) Outbreak and control of dengue in a village in Dharmapuri, Tamil Nadu. *Journal of Communicable Disease* **29**, 69–71.

Carey DE, Myers RM & Reuben R (1964) Dengue types 1 & 4 viruses in wild – caught mosquitoes in south India. *Science* **143**, 131–132.

Chen WJ, Chen SL, Chein LJ *et al.* (1996) Silent transmission of the dengue virus in southern Taiwan. *American Journal of Tropical Medicine and Hygiene* **55**, 12–16.

Cherian T, Ponnuraj E, Kuruvilla T, Kirubakaran C, John TJ & Raghupathy P (1994) An epidemic of dengue haemorrhagic fever and dengue shock syndrome in and around Vellore. *Indian Journal of Medical Research* **100**, 51–56.

Chow VTK, Chan YC, Yong R *et al.* (1998) Monitoring of dengue viruses in field-caught *Aedes aegypti* and *Aedes albopictus* mosquitoes by a type-specific polymerize chain reaction and cycle sequencing. *American Journal of Tropical Medicine and Hygiene* **58**, 578–586.

Diallo M, Yamar B, Sall AA *et al.* (2003) Amplification of the sylvatic cycle of dengue virus type 2, Senegal 1999–2000: entomologic findings and epidemiologic considerations. *Emerging Infectious Diseases* **9**, 362–366.

Endy TP, Chunsuttiwat S, Nisalak A *et al.* (2002) Epidemiology of inapparent and symptomatic acute dengue virus infection: a prospective study of primary school children in Kamphaeng Phet, Thailand. *American Journal of Epidemiology* **156**, 40–51.

Focks DA & Chadee DD (1997) Pupal survey: an epidemiologically significant surveillance method for *Aedes aegypti*: an example using data from Trinidad. *American Journal of Tropical Medicine and Hygiene* **56**, 159–167.

Focks DA, Brenner RJ, Jack Hayer & Daniels E (2000) Transmission of thresholds for dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in some source reduction efforts. *American Journal of Tropical Medicine and Hygiene* **62**, 11–18.

Gajanana A, Rajendran R, Thenmozhi V, Philip Samuel P, Tsai TF & Reuben R (1995a) Comparative evaluation of bioassay and ELISA for detection of Japanese encephalitis virus in field collected mosquitoes. *Southeast Asian Journal of Tropical Medicine and Public Health* **26**, 91–97.

Gajanana A, Thenmozhi V, Philip Samuel P & Reuben R (1995b) A community-based study of subclinical flavivirus infections in children in an area of Tamil Nadu, India, where Japanese encephalitis is endemic. *Bulletin of World Health Organization* **73**, 237–244.

Gubler DJ (1997) Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. In: *Dengue and Dengue Haemorrhagic Fever* (eds DJ Gubler & G Kuno) CAB International, London, pp. 1–22.

Gubler DJ (1998) Dengue and dengue hemorrhagic fever. *Clinical Microbiology Reviews* **11**, 480–496.

Gubler DJ, Reed D, Rosen L & Hitchcock (1978) Epidemiologic, clinical, and virologic observations on dengue in the Kingdom of Tonga. *American Journal of Tropical Medicine and Hygiene* **27**, 581–589.

Halstead SB (1980) Dengue hemorrhagic fever – public health problem and a field for research. *Bulletin of World Health Organization* **58**, 1–21.

Halstead SB (1994) Dengue and dengue hemorrhagic fever. In: *Handbook of Zoonoses*, 2nd edn (eds GW Beran & JH Steele) CRC Press, Boca Raton, FL, pp. 89–99.

Hawley WA (1988) The biology of *Aedes albopictus*. *Journal of American Mosquito Control Association* **4**, 2–39.

Ilkal MA, Dhanda V, Hassan MM *et al.* (1991) Entomological investigations during outbreaks of dengue fever in certain villages in Maharashtra state. *Indian Journal of Medical Research* **93**, 174–178.

Joshi PT, Pandya AP & Anjan JK (2000) Epidemiological and entomological investigation in dengue outbreak area of Ahmedabad district. *Journal of Communicable Disease* **32**, 22–27.

Kabra SK, Verma IC, Arora NK, Jain Y & Kalra V (1992) Dengue haemorrhagic fever in children in Delhi. *Bulletin of the World Health Organization* **70**, 105–108.

Katholi, CR, Toe L, Merriweather A & Unnasch TR (1995) Determining the prevalence of *Onchocerca volvulus* infection in vector populations by PCR screening of pools of black flies. *Journal of Infectious Disease* **172**, 1414–1417.

Lam SK (1993) Rapid dengue diagnosis and interpretation. *Malaysian Journal of Pathology* **15**, 9–12.

Mahadev PVM, Kollali VV, Rawal ML *et al.* (1993) Dengue in Gujarat state, India during 1988 & 1989. *Indian Journal of Medical Research* **97**, 135–144.

Myers RM, Carey DE, DeRantiz CM, Reuben R & Bennet B (1969) Virological investigations of the 1966 outbreak of dengue type 3 in Vellore, southern India. *Indian Journal of Medical Research* **57**, 1392–1401.

Myers RM, Varkey MJ, Reuben R & Jesudass ES (1970) Dengue outbreak in Vellore, southern India, in 1968, with isolation of four dengue types from man and mosquitoes. *Indian Journal of Medical Research* **58**, 24–30.

Norman G, Theodre A & Joseph A (1991) An insular outbreak of dengue fever in a rural South Indian village. *Journal of Communicable Disease* **23**, 185–190.

Reiter P & Gubler DJ (1997) Surveillance and control of urban dengue vectors. In: *Dengue and Dengue Hemorrhagic Fever*

(eds DJ Gubler & G Kuno) CAB International, London, pp. 425–462.

Reuben R (1971a) Studies on the mosquitoes of North Arcot district, Madras state, India. *Journal of Medical Entomology* **8**, 119–126.

Reuben R (1971b). Studies on the mosquitoes of North Arcot district, Madras state, India. *Journal of Medical Entomology* **8**, 258–262.

Reuben R & Panickar KN (1975) *Aedes* survey in five districts of Rajasthan, India. *Journal of Communicable Diseases* **7**, 1–9.

Reuben R, Kaul HN & Soman RS (1988). Mosquitoes of arboviral importance in India. *Mosquito Borne Disease Bulletin* **5**, 48–54.

Reuben R, Thenmozhi V, Samuel PP, Gajanana A & Mani TR (1992) Mosquito blood feeding patterns as a factor in the epidemiology of Japanese encephalitis in southern India. *American Journal of Tropical Medicine and Hygiene* **6**, 654–663.

Rigau-Perez JG, Clark GG, Gubler DJ, Reiter P, Sanders EJ & Vorndam AV (1998) Dengue and dengue haemorrhagic fever. *Lancet* **352**, 971–977.

Rodhain F & Rosen L (1997). Mosquito vectors and DENV–vector relationships. In: *Dengue Hemorrhagic Fever* (eds DJ Gubler & G Kuno) CAB International, London, pp. 45–60.

Rodrigues SME, Dai-Fabbro AL, Salomao R, Ferreira IB, Rocco IM & Lopes da Fonseca BA (2002) Epidemiology of dengue infection in Ribeirao Preto, SP, Brazil. *Revista de Saude Publica* **36**, 160–165.

Sabin AB (1952) Research on dengue during World War II. *American Journal of Tropical Medicine and Hygiene* **1**, 30–50.

Sheppard PM, Macdonald WW & Tonn RJ (1969) A new method of measuring the relative prevalence of *Aedes aegypti*. *Bulletin of World Health Organization* **40**, 467.

Singh J, Balakrishnan N, Bhardwarj M *et al.* (2000) Silent spread of dengue and dengue haemorrhagic fever to Coimbatore and Erode districts in Tamil Nadu, India 1998: need for effective surveillance to monitor and control the disease. *Epidemiology and Infection* **125**, 195–200.

Teixeira MG, Barreto ML, Costa MCN, Ferreira LDA, Vasconcelos PFC & Cairncross S (2002) Dynamics of dengue virus circulation: a silent epidemic in a complex urban area. *Tropical Medicine and International Health* **9**, 757–762.

Thavara U, Tawatsin A, Chansang C *et al.* (2001) Larval occurrence, oviposition behaviour and biting activity of potential mosquito vectors of dengue on Samui Island, Thailand. *Journal of Vector Ecology* **26**, 172–180.

Thenmozhi V, Tewari SC, Manavalan R, Balasubramanian A & Gajanana A (2000) Natural vertical transmission of dengue viruses in *Aedes aegypti* in southern India. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **94**, 507.

Tidwell MA, Williams DC, Tidwell TC *et al.* (1990) Baseline data on *Aedes aegypti* populations in Santo Domingo, Dominican Republic. *Journal of American Mosquito Control Association* **6**, 514–522.

Victor TJ, Malathi M, Gurusamy D *et al.* (2002) Dengue fever outbreaks in two villages of Dharmapuri district in Tamil Nadu. *Indian Journal of Medical Research* **116**, 133–139.

Vijayarani H & Gajanana A (2000) Low rate of Japanese encephalitis infection in rural children in Thanjavur district (Tamil Nadu), an area with extensive paddy cultivation. *Indian Journal of Medical Research* **111**, 212–214.

WHO (1997) *Dengue Haemorrhagic Fever: Diagnosis, Treatment, Prevention and Control*, 2nd edn. World Health Organization, Geneva, pp. 1–84.

WHO (1999) *Prevention and Control of Dengue and Dengue Haemorrhagic Fever: Comprehensive Guidelines*. WHO Regional Publications, SEARO No. 29.

Authors

S. C. Tewari (corresponding author), **V. Thenmozhi, R. Manavalan** and **A. Munirathinam**, Centre for Research in Medical Entomology, Indian Council of Medical Research, 4 Sarojini Street, Chinna Chokkikulam, Madurai 625 002, India. Tel.: +91 452 2650281; Fax: +91 452 2530660; E-mail: crmeicmr@satyam.net.in

C. R. Katholi, Department of Biostatistics, University of Alabama at Birmingham, 1665 University Blvd., Birmingham, AL 35294, USA. E-mail: ckatholi@uab.edu

A. Gajanana, 146, 11th Main Road, Hanumantha Nagar, Bangalore 560 019, India. E-mail: gajanana_a@hotmail.com