

Molecular characterization of extrinsic peri-urban dengue viruses contributing to cause DHF: A new concept for mechanism of DHF - *Vinod Joshi, Bennet Angel and Rashmi Chauhan*

Commencement: **December, 2005**

Duration: **Three Years**

Status: **Ongoing**

Objectives

1. To confirm occurrence of Zoonotic cycle (ZC) of Dengue virus among tree hole breeding mosquitoes and inhabiting monkey populations. Study of its occurrence and epidemiological significance in desert settings of Rajasthan.
2. Study of role of Zoonotic cycle in maintaining and/or amplifying endemic cycle of dengue fever.
3. Study of possible role of Zoonotic cycle as factor causing Dengue Hemorrhagic Fever.
4. To undertake experimental studies to confirm monkey- mosquito cycle of dengue in laboratory models of monkeys.

Rationale

DHF is the stage believed to be the result of failure of immune response of a human host towards a new dengue serotype when responding host is already immune against another dengue strain to which it was exposed previously (Halstead, 2002). At present it is being believed that active dengue cases infected of a strain different than previous strain, are the sources of introducing second strain to cause DHF in an area.

Dengue viruses are the members of flaviviruses which are highly antigenic. Owing to this, net viremia available in human blood is around 10^5 /ml of blood only. With this stock of virus which is a challenged stock by immune system of humans, an infected human may not be able to provide sufficient unchallenged dengue antigen to a previously exposed case of DF to cause DHF. We believe that to cause DHF humans may be a dead end as they are in the case of arbo-virus Japanese encephalitis. It is perhaps for this reason that in Delhi appearance of DEN-2 in 1996 and now appearance of DEN-4 in 2006 have not led to an epidemic of DHF. In Africa also with a gap of 10 years two strains of dengue infected the population, no DHF could be reported. We believe that for providing viraemically strong virus stock, a mosquito passaged virus in peri-urban foci, can only cause DHF. We thus need to type and sequence the virus of DHF patients and match sequences with available peri-urban viruses within mosquitoes to prove this hypothesis.

In support of above hypothesis it is mentioned here that dengue viruses were primarily the viruses of mosquitoes before they adapted to lower primates. We believe that crude and undisturbed ecological niches of peri-urban or sylvatic foci of dengue maintained within mosquitoes of zoos/parks or within larval mosquito pools breeding in permanent and protected reservoirs in city setup do not get weakened titre for less feeding opportunity available with the tree hole breeders to feed upon challenging systems of humans. If strains contained by mosquitoes of peri-urban foci happen to be different than urban strain, mixing of two strains may straight be causal for the DHF. In such case, proposed research will pave a very effective prevention strategy against DHF. To pursue this contention, present studies have been pursued.

Progress of the work

Study areas

Present understanding of etiology of Dengue Hemorrhagic Fever (DHF) is based on cross reactivity of two dengue strains appearing in a common host with a gap of few months/years of infectivity from each other. Keeping this in view, it is presumed that endemic dengue has a uniform infectivity by one DEN strain and that zoonotic or sylvatic foci of virus between tree hole breeding *Aedes albopictus* and monkeys or through vertically transmitted virus within generations of *Aedes albopictus*, introduce another DEN strain to endemic population. To study above concept we have undertaken typing of mosquito viruses from 4 endemic areas of Rajasthan as shown in the Table 1. Mosquito contained dengue strains from urban, rural and peri-urban foci of Jodhpur have been typed from December 2006 to February, 2008 (Table 2).

Observations on distribution of various virus types in endemic settings of Rajasthan.

- Among urban setting of desert area, all the four dengue types viz; DEN-1, 2, 3 and 4 were detected. Maximum presence (37.1 %) of DEN -2 and DEN-3 (31.4 %) were observed, while DEN-1 and DEN-4 were relatively less prevalent (14.2 and 11.4 % respectively). In peri-urban setting of desert area DEN-3 was observed whereas, among rural settings DEN-2 was detected.
- In semi-arid area, urban settings showed presence of DEN-3, whereas among rural settings of this area, rural-1 showed all the four DEN types present, rural-2 showed DEN-1 and DEN-3, rural-4 showed DEN-3 and DEN-4 and rural-3 showed no mosquito infections.
- In forest & river area, among urban settings, three DEN types, 1,2 and 4 were observed, whereas, DEN-3 was not observed from these settings. Among rural settings of this area, in rural-1, only DEN-2 was present, in rural-2, DEN-1 and DEN-4 were present.

- The data show that while in desert and semi-arid areas, all the four DEN types were observed in urban settings, whereas in forest & river area, DEN-3 was not observed from any of the settings screened. In non-arid area no mosquito infection was observed. Pooled data suggest that in urban settings 34.1 % and rural settings 36.3 % mosquitoes, maximum presence of DEN-2 was observed followed by DEN-3.

Inferences on distribution of various mosquito types in endemic settings of Rajasthan

- In desert and semi-arid areas of Rajasthan, where people show tendency storage of domestic water, present observations on occurrence of all four dengue strains, would ensure presence of different DEN types and thus present observations may have important bearing on the possibility of emergence of DHF in the area. It was interesting to observe that in both desert as well semi-arid areas, among urban settings, all the four dengue types were observed in the field collected mosquitoes but in forest and river area DEN-3 was not detected in any of the mosquito samples. These observations highlight specificity of areas for maintaining particular DEN types.
- In the present study, the mosquito infections detected through IFAT and their subsequent confirmation in cell culture medium, mouse brain as the possible screening tool to estimate risk of DHF in an area. The approach of virus typing in field collected mosquitoes could yield a powerful surveillance indicator to investigate current scenario of virus types available and develop predictors of the prospective risk of occurrence of DHF.
- The antigenic information on the virus types contained by extrinsic sources may be helpful in designing the surveillance of dengue.

Work remains to be done

We are pursuing typing of virus among tree hole breeding *Aedes albopictus*. Subsequently we propose to sequence the genome of viral strain causing DHF in human and match it with genome of *Aedes albopictus* contained virus.

Table 1. Distribution of dengue types in *Aedes aegypti* of four endemic areas of Rajasthan

Area	Rural/Urban	Total tested	Total positive	% +ve	+ve DE N-1	% +ve	+ve DE N-2	% +ve	+ve DE N-3	% +ve	+ve DE N-4	% +ve
Desert Area	Urban setting	162	35	21.6	5	14.2	13	37.1	11	31.4	6	11.4
	Peri-urban setting	11	1	9.0	0	0.0	0	0.0	1	100.0	0	0.0
	Rural setting	16	4	25.0	0	0.0	4	100.0	0	0.0	0	0.0
Semi-arid Area	Urban setting	31	1	3.2	0	0.0	0	0.0	1	100.0	0	0.0
	Rural setting -1	62	15	24.1	4	26.6	5	33.3	3	20.0	3	20.0
	Rural setting-II	32	4	12.5	2	50.0	0	0.0	2	50.0	0	0.0
	Rural setting-III	4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
	Rural setting-IV	26	3	11.5	0	0.0	0	0.0	2	66.6	1	33.3
Forest & River Area	Urban setting	70	5	7.1	3	60.0	1	20.0	0	0.0	1	20.0
	Rural setting-I	27	3	11.1	0	0.0	3	100.0	0	0.0	0	0.0
	Rural setting-II	36	4	11.1	1	25.0	0	0.0	0	0.0	3	75.0
Non-arid Area	Urban setting	21	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
Total	Urban	284	41	14.4	11	26.8	14	34.1	12	29.2	7	17.0
	Peri-urban	11	1	9.0	0	0.0	0	0.0	1	100.0	0	0.0
	Rural	203	33	16.2	7	21.2	12	36.3	7	21.2	7	21.2
Pooled	Setting	498	75	15.0	18	24.0	26	34.6	20	26.6	14	18.6

Table 2. Virus typing in urban, peri-urban and rural settings of Jodhpur

Month		Urban			Peri-urban			Rural			Total		
		Ae.	Ae.	Ae.	Ae.	Ae.	Ae.	Ae.	Ae.	Ae.	Ae.	Ae.	Ae.
Jan.	Total	106	X	-	-	-	-	25	X	-	131	-	-
	Positive	25	X	-	-	-	-	13	X	-	38	-	-
	% positive	23.5	X	-	-	-	-	52.0	X	-	29.0	-	-
Feb.	Total	92	X	-	13	2	-	72	X	-	177	2	-
	Positive	21	X	-	0	0	-	26	X	-	47	0	-
	% positive	22.8	X	-	0	0	-	36.1	X	-	26.5	0	-
Mar.	Total	-	X	-	3	3	-	-	X	-	3	3	-
	Positive	-	X	-	0	0	-	-	X	-	0	0	-
	% positive	-	X	-	0	0	-	-	X	-	0	0	-
Apr.	Total	50	X	-	4	20	3	27	X	58	81	20	61
	Positive	5	X	-	0	1	0	3	X	0	8	1	0
	% positive	10	X	-	0	5.0	0	11.1	X	0	9.87	5.0	0
May	Total	64	X	-	8	2	11	64	X	18	136	2	11
	Positive	11	X	-	0	0	0	10	X	0	21	0	0
	% positive	17.1	X	-	0	0	0	15.6	X	0	15.4	0	0
June	Total	8	X	-	-	-	5	-	X	6	8	-	11
	Positive	1	X	-	-	-	0	-	X	0	1	-	0
	% positive	12.5	X	-	-	-	0	-	X	0	12.5	-	0
July	Total	-	X	-	-	-	6	-	X	-	-	-	6
	Positive	-	X	-	-	-	0	-	X	-	-	-	0
	% positive	-	X	-	-	-	0	-	X	-	-	-	0
Aug.	Total	51	X	22	4	48	-	-	X	9	55	48	31
	Positive	16	X	0	0	3	-	-	X	0	16	3	0
	% positive	31.3	X	0	0	6.25	-	-	X	0	29.0	6.25	0
Sept.	Total	38	X	-	2	17	25	2	X	20	42	17	45
	Positive	12	X	-	0	1	1	0	X	2	12	1	3
	% positive	31.5	X	-	0	5.88	4.0	0	X	10.0	28.5	5.88	6.66
Oct.	Total	84	X	22	-	48	30	16	X	-	100	48	52
	Positive	13	X	2	-	9	3	3	X	-	16	9	5
	% positive	15.4	X	9.09	-	18.75	10.0	18.7	X	-	16.0	18.7	9.61
Nov.	Total	23	X	46	-	47	-	11	X	-	34	47	46
	Positive	8	X	10	-	13	-	0	X	-	8	13	10
	% positive	34.7	X	21.7	-	27.6	-	0	X	-	23.5	27.6	21.7
Dec.	Total	-	X	-	-	45	-	36	X	-	36	45	-
	Positive	-	X	-	-	21	-	11	X	-	11	21	-
	% positive	-	X	-	-	46.6	-	30.5	X	-	30.5	46.6	-
Total	Total	508	X	90	34	227	80	253	X	111			
	Positive	111	X	12	0	48	4	66	X	2			
	% positive	21.8	X	13.3	0	21.1	13.3	26.0	X	1.80			

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