

## 1.2 Mapping of risk of Dengue Hemorrhagic Fever (DHF) through dengue virus typing in *Aedes* mosquitoes of different settings of Rajasthan-

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**Commencement:** May, 2012

**Duration:** Two Years

**Status:** Ongoing

**Funding agency:** Indian Council of Medical Research (Extramural)

### PROGRESS

#### 1.1. Field work undertaken:

During the reported period of 10 months (from May, 2012 till March, 2013), total number of 12 different districts have been covered for the collection of mosquito immatures. The districts of Jalore, Pali, Barmer, Sirohi, Jaisalmer, Bikaner, Churu, Ganganagar, Hanumangarh, Nagaur, Ajmer and Udaipur have been covered (Fig. 1).



Fig. 1. Study villages covered during reported period

All the domestic as well as peri-domestic containers available in a selected house were searched for *Aedes* breeding. Total number of systematically sampled 6275 houses were screened in these districts of which 7.44% were positive for the breeding of *Aedes* mosquitoes. *Aedes* larvae were collected from containers found positive for breeding which were then brought to the laboratory for virus isolation studies. Of the total 38,199 containers surveyed, 552 containers were found to be positive. The key containers observed positive for *Aedes* breeding were cemented tanks and clay pots. The details of entomological observations are depicted in Table 1.

**Table 1. Details of entomological investigations in study villages**

Districts covered	Total houses surveyed	Total houses +ve for breeding	% +ve	Total containers examined	Total containers +ve for breeding
Jalore	307	29	9.4	2346	40
Pali	325	105	32.3	2365	147
Sirohi	410	26	6.34	2487	25
Barmer	417	13	3.11	2568	16
Jaisalmer	294	6	2.04	1711	7
Bikaner	996	97	9.7	5999	106
Churu	246	7	2.8	1417	7
Ganganagar	555	70	12.6	3180	73
Hanumangarh	343	21	6.12	1677	21
Nagaur	1017	62	6.09	7234	79
Ajmer	676	31	4.58	4523	31
Udaipur	689	0	0	2692	0
<b>Total</b>	<b>6275</b>	<b>467</b>	<b>7.44</b>	<b>38199</b>	<b>552</b>

## 1.2. Virus isolations from field collected and laboratory reared larvae:

The field collected larvae were reared into adult mosquitoes at DMRC laboratory. Adults were subjected to Indirect Fluorescence Antibody Test (IFAT) to detect presence of dengue virus. Of 6275 houses surveyed in 12 districts, 467 were found positive for breeding of *Aedes* mosquitoes. Of these, 307 (4.89%) houses were positive for dengue virus presence. Percentage of houses positive for dengue virus ranged from 25.8% in Pali district to 1.1% in Hanumangarh district. In 6275 houses, 38199 containers were examined for breeding of which 329 (0.86%) were positive for the presence of dengue virus. The percentage of virus positive containers was 0.2% in Hanumangarh district to 3.9% in Pali district (Table 2).

From 6275 houses surveyed and 38199 containers examined, 1053 larvae were collected and reared into adult mosquitoes. Maximum mosquito infections were observed in Ajmer district, where 38.6% laboratory reared adults showed presence of virus where as least mosquito infectivity was observed in Hanumangarh district where 0.2 % infectivity was observed. No mosquito infections were observed in Udaipur, and Jaisalmer districts (Table 3).

**Table 2. Details of virus isolations from laboratory reared *Aedes* mosquitoes in study villages**

Districts covered	Total houses surveyed	Total houses +ve for breeding	Total houses +ve for virus	% of virus +ve Houses	Total containers examined	Containers +ve for virus	% virus +ve containers
Jalore	307	29	19	6.18	2346	20	0.85
Pali	325	105	84	25.84	2365	94	3.97
Sirohi	410	26	20	4.87	2487	20	0.80
Barmer	417	13	10	2.39	2568	11	0.42
Jaisalmer	294	6	0	0	1711	0	0
Bikaner	996	97	57	5.72	5999	59	0.98
Churu	246	7	7	2.84	1417	7	0.49
Ganganagar	555	70	52	9.36	3180	56	1.76
Hanumangarh	343	21	4	1.16	1677	4	0.23
Nagaur	1017	62	39	3.83	7234	43	0.59
Ajmer	676	31	15	2.21	4523	15	0.33
Udaipur	689	0	0	0	2692	0	0
<b>Total</b>	<b>6275</b>	<b>467</b>	<b>307</b>	<b>4.89</b>	<b>38199</b>	<b>329</b>	<b>0.86</b>

**Table 3. Details of virus isolations from individual adult mosquitoes**

Districts covered	Total mosquitoes assayed	Mosquitoes +ve for dengue virus	% +ve
Jalore	60	12	20
Pali	205	56	27.31
Sirohi	75	23	30.66
Barmer	50	9	18
Jaisalmer	20	0	0
Bikaner	200	41	20.5
Churu	40	12	30
Ganganagar	145	34	23.44
Hanumangarh	43	3	6.97
Nagaur	140	34	24.28
Ajmer	75	29	38.66
Udaipur	0	0	0
<b>Total</b>	<b>1053</b>	<b>253</b>	<b>24.02</b>

The mosquitoes observed positive for IFAT (Fig. 1) were subjected to Real Time PCR (RT-PCR) (Fig. 2) to confirm the virus isolations as observed through IFAT. Type specific virus isolations were made from the field collected and laboratory reared mosquitoes collected from Pali, Bikaner and Barmer districts. The pilot run of type specific virus isolations from sub samples of mosquitoes, has been done using monoclonal antibodies employing IFAT.

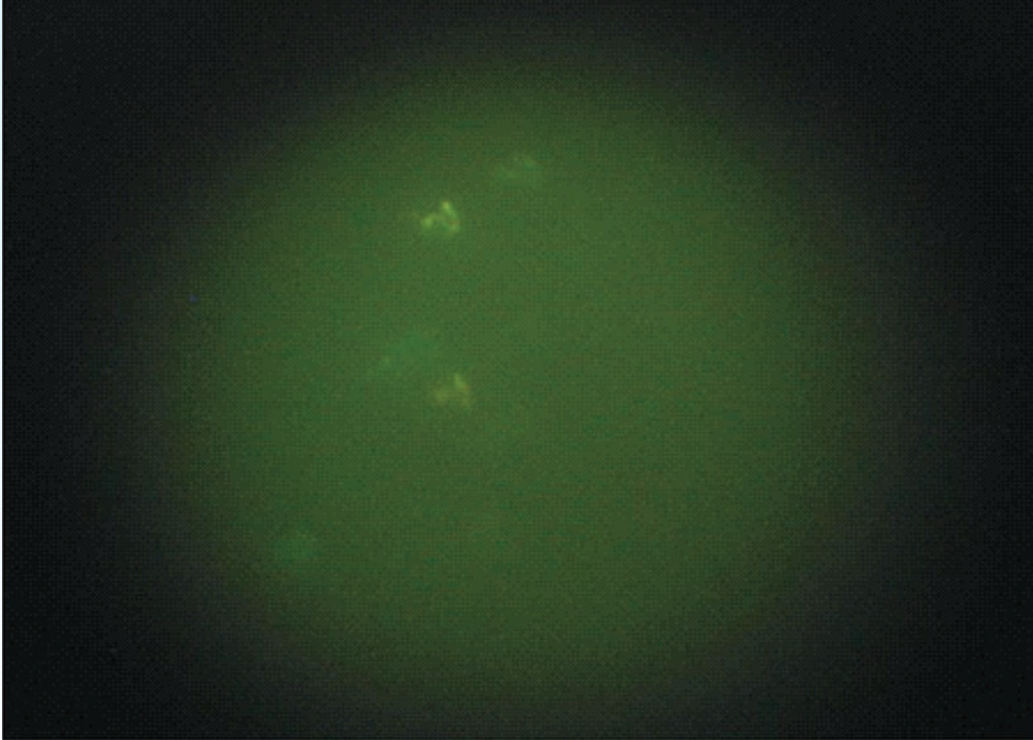


Fig. 1. Virus positivity as viewed employing IFAT

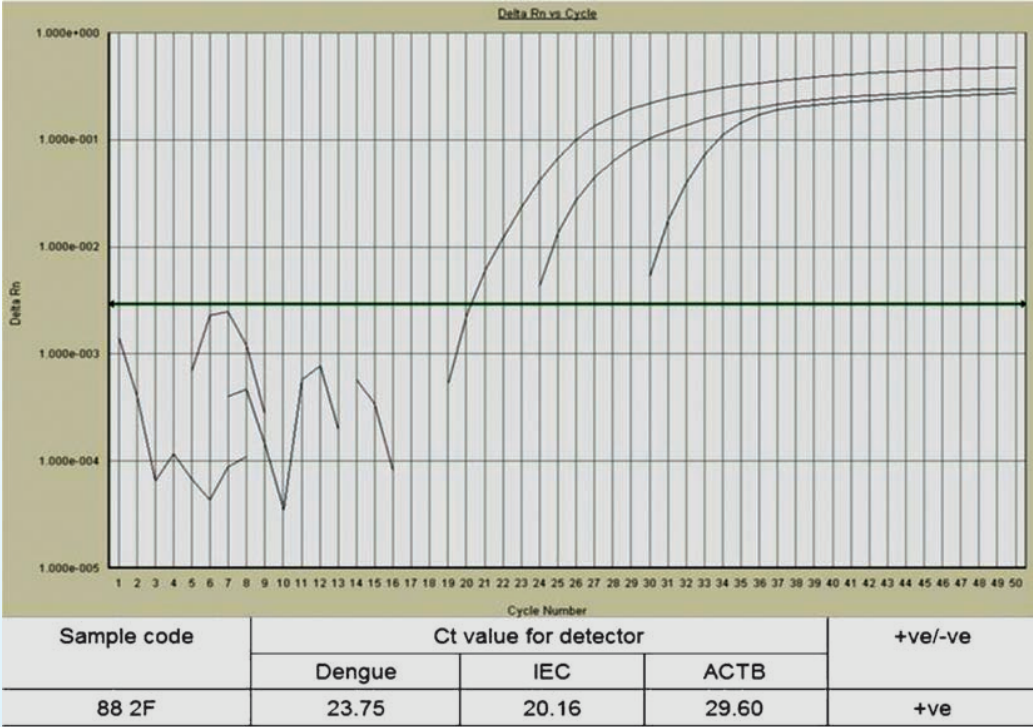


Fig. 2. Virus positivity as confirmed employing Real Time PCR (RT-PCR)

All the samples consisted of two *Aedes* species viz; *Aedes aegypti* and *Aedes vittatus*. DEN-1, DEN-2, DEN-3 and DEN-4 virus strains were detected in individual mosquitoes as trial run of type specific virus isolations. *Aedes aegypti* showed 17.9% DEN-1, 23.2% DEN-2, 40.2% DEN-3 and 19.4% DEN-4. DEN-3 strain was observed maximum in *Aedes aegypti*. Among *Aedes vittatus* subjected to IFAT, maximum presence (37.7%) of DEN-2 was observed (Table 4).

**Table 4. DEN type specific virus isolations from mosquito samples in Pali, Barmer and Bikaner districts**

Species	Total tested	Total positive	% positive	Positive for DEN-1 (%)	Positive for DEN-2 (%)	Positive for DEN-3 (%)	Positive for DEN-4 (%)
<i>Aedes aegypti</i>	299	67	22.40	12 (17.9)	15 (22.4)	27 (40.3)	13 (19.4)
<i>Aedes vittatus</i>	151	45	29.80	9 (20.0)	17 (37.8)	14 (31.1)	5 (11.1)
<b>Total</b>	450	112	24.88	21 (18.8)	32 (28.6)	41 (36.6)	18 (16.1)

All the mosquito samples reared in laboratory have been pooled district-wise and stored at -75°C. The samples have been subjected for molecular isolation using Real Time PCR.

### 1.3. Summary of work:

1. Of the 32 districts selected for study, 12 districts have been covered during reported period. Field collected larvae have been reared into adults, mosquitoes have been subjected to IFAT and screening of virus positive mosquitoes has been made.
2. Typing of mosquito samples has been standardized using monoclonal antibodies employing IFAT.
3. Selected samples of all the IFAT positive mosquitoes have been subjected to virus isolation through Real Time PCR and positive samples have been stored at -75°C.
4. Serum samples have been collected from suspected cases of dengue from hospitals and to confirm the positivity of samples, all have been tested employing Mac-ELISA. The samples have been stored at -75°C for DEN type specific Real Time PCR.
5. Mosquito samples of 12 districts have been processed for virus isolations employing RT-PCR.
6. After RNA extractions samples have been stored for further assay of DEN type specific isolation using RT-PCR.