J Vector Borne Dis 45, March 2008, pp. 56–59

Distribution and seasonality of vertically transmitted dengue viruses in *Aedes* mosquitoes in arid and semi-arid areas of Rajasthan, India

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Abstract

Background & objectives: Transovarial transmission of dengue virus is a crucial etiological phenomenon responsible for persistence of virus during inter-epidemic period of the disease. Distribution and seasonality of this phenomenon in disease endemic areas may contribute to explain emergence of dengue and its subsequent prevention. The study on seasonal and area distribution of transovarial transmission of dengue virus in *Aedes aegypti*, *Ae. albopictus* and *Ae. vittatus* has been made in desert and non-desert districts of Rajasthan, India from 2006 to 2007. The observations revealed role of different *Aedes* species in transmission and retention of dengue virus.

Methods: The larvae of *Ae. aegypti, Ae. albopictus* and *Ae. vittatus* were collected during each of the study seasons from rural and urban areas of three districts—Jodhpur, Jaipur and Kota. The larvae were collected from domestic and peri-domestic containers and from tree holes of peri-urban foci such as gardens and parks and were reared into adults in the laboratory at room temperature. The laboratory reared adults were subjected to Indirect fluorescence antibody test (IFAT). The laboratory-reared adult mosquitoes showing positive IFA were treated as the sample showing vertically transmitted dengue virus.

Results: Pooled data for all the four seasons revealed maximum (15.7%) mosquito infectivity in *Ae. albopictus* followed by *Ae. aegypti* (12.6%) in Jodhpur district. In Jaipur district, *Ae. vittatus* showed highest infection (20%) of vertically transmitted virus followed by *Ae. albopictus* (18.7%) and least in *Ae. aegypti* (13.3%). In Kota district, pooled data for all the four seasons showed maximum vertical infection of mosquitoes in *Ae. albopictus* (14.2%).

Interpretation & conclusion: Transovarial transmission of dengue virus by available vector species in a dengue endemic setting could be the key etiological phenomenon responsible for re-emergence of the disease from inter-epidemic to epidemic phase of disease onset. The observations in the present study suggest that during winter season which is not the active transmission season of dengue in Rajasthan, *Ae. albopictus* has shown maximum percentage of vertically transmitted virus. Our observation substantiates with the earlier studies that how *Ae. albopictus* is horbouring virus during inter-epidemic period of dengue. Another important lead emerging through present study is the high mosquito infectivity of *Ae. aegypti* during summer and rainy seasons especially from desert districts, Jodhpur and semi district. This observation suggests that in Rajasthan, owing to tendency of overstorage of domestic water by the inhabitants, mosquito and vertically transmitted virus get pronounced during summer season which could precedes the active transmission season of dengue during following rainy season.

Key words *Aedes* – India – transovarial transmission

Introduction

Persistence of vertical or transovarial transmission of dengue viruses may serve to retain dengue viral pathogen in nature during inter-epidemic periods of the disease[1]. Presence of this virus stock within the mosquito fauna surviving during non-epidemic periods of disease, in fact, may be a possible cause of re-emergence of dengue in an area previously exposed to dengue. Thus monitoring natural infectivity of mosquitoes carried through vertical route of virus, may serve as an important surveillance tool for risk prediction as well as for the prevention of dengue emergence in an endemic setting. This surveillance index has not been employed yet to estimate the risk of dengue emergence in an area and pin point vector-virus foci for their elimination during non-epidemic periods of dengue. Present paper reports results of a comprehensive study on distribution and seasonality of vertical transmission of dengue viruses by *Aedes aegypti*, *Ae. albopictus* and *Ae. vittatus* (Diptera: Culicidae) mosquitoes in the rural and urban areas of three districts of Rajasthan, India, from 2006 to 2007.

Material & Methods

Study areas: Jodhpur district comprises of plain sandy terrain with scanty or thin vegetation. Due to very few monsoon rains and scarcity of natural water resources the human population in this area is sparse. The inhabitants of this region have habits of storing water in large number of containers ranging from clay pots, cement tanks, plastic containers, underground water storage, etc. especially during the summer season. Majority of these containers are ad-hoc reservoirs owing to the low economic status of the families.

Jaipur district basically consists of foothills. The ecology of the area is somewhat like a semi-arid setting. The population residing here shows cosmopolitan character. The inhabitants by and large exhibit mixed sociocultural setup. The natural vegetation and water resources are not very abundant but due to urbanization, sufficient water and developed vegetation is available.

Kota district consists of relatively more forests and marked by the presence of rivers. The area is often flooded in rains, therefore, has ample amount of water available throughout monsoon season resulting into natural water resources with enough water. The region also consists of large industries which influence the social setup of the region.

Collection and laboratory rearing of larvae and virus isolation from adult mosquitoes: The larvae of *Ae. aegypti, Ae. albopictus* and *Ae. vittatus* were collected during each of the study seasons—winter, spring, summer and rainy from rural and urban areas of three districts namely, Jodhpur, Jaipur and Kota. The larvae were collected from domestic and peri-domestic containers and from tree holes of peri-urban foci such as gardens and parks. The larvae collected through sieve were brought to the laboratory in plastic bottles and were reared into adults in laboratory at room temperature. The adults reared in laboratory were kept in Barraud cages on 4% glucose solution for 2–3 days to allow the development of virus, if present in them. The laboratory reared adults were subjected to indirect fluorescence antibody test (IFAT) following standard protocol[2]. The laboratory-reared adult mosquitoes showing positive IFAT were treated as the sample showing vertically transmitted dengue virus. High titrated immune serum raised in rabbits against dengue viruses at the National Institute of Virology, Pune, India, was used as a source of dengue antibodies. Fluorescene isothyocynate (FITC) used for assay was procured from M/s. Sigma, USA. Fluorescence microscope model BH2 RFL1 PM 10 ADS, made by M/s. Olympus, Japan was used to view the detection of virus as fluorescence.

To avoid possible bias in examining IFAT slides, negative controls (laboratory-reared mosquitoes from areas other than study areas, showing no fluorescence), positive controls (laboratory-reared mosqui-

toes from areas other than study areas, showing fluorescence) were shown to blinded observer to match the unbiased observations for IFAT positive and negative slides. Remnants of mosquitoes showing positive IFAT, were used for preparing suspension in bovine albumin. About 22 µl of suspension was intra-cerebrally inoculated into infant albino mice (2-days old). The mice which developed sickness, were sacrificed in their last stage of activity, brain was dissected out and used to prepare virus suspension in phosphate buffer saline (PBS). This was again subjected to IFAT to confirm the presence of virus.

Results

Pooled data for all the four seasons in Jodhpur district revealed maximum (15.7%) mosquito infection in *Ae. albopictus* followed by *Ae. aegypti* (12.6%) and *Ae. vittatus* (5.14%). Seasonal split of observations indicate same trend in winter and summer seasons, whereas in rainy season *Ae. aegypti* showed maximum vertically infected mosquitoes followed by *Ae. albopictus* (12.2%).

In Jaipur district, data pooled for all the four seasons showed highest (20%) infection of vertically transmitted virus in *Ae. vittatus*, followed by *Ae. albopictus* (18.7%) and least in *Ae. aegypti* (13.3%). In individual seasons, rainy season showed similar trend — maximum mosquito infection of *Ae. vittatus* (20.4%) followed by *Ae. albopictus* (18.7%). However, in summer and winter seasons only *Ae. aegypti* showed vertically transmitted virus (Table 1). In Kota district, pooled data for all the four seasons showed maximum vertical infection of mosquitoes in *Ae. albopictus* (14.2%) followed by *Ae. aegypti* (7.07%) and *Ae. vittatus* (4.76%). However, in individual seasons *Ae. aegypti* showed maximum transovarial transmission of dengue virus (Table 1).

Discussion

Transovarial transmission of dengue virus by available vector species in a dengue endemic setting could be the key etiological phenomenon responsible for re-emergence of disease from inter-epidemic to epidemic phase of disease onset. In addition, during an active outbreak of disease this route of virus transmission could also be supplementing to the population of infected mosquitoes resulting from horizontal trans-

Table 1. Seasonal and area specific distribution of transovarial transmission of dengue virus by *Aedes* mosquitoes in Rajasthan, India

Area	Species	Winter		Spring		Summer		Rainy		Pooled	
		No. examined	No. +ve (%)	No. examined	No. +ve (%)	No. examined	No. +ve (%)	No. examined	No. +ve (%)	No. examined	No. +ve (%)
Jodhpur	*Ae. aegypti*	67	9 (13.4)	84	0	184	29 (15.8)	133	21 (15.8)	468	59 (12.6)
	Ae. albopictus	43	9 (20.9)	15	0	64	14 (21.9)	106	13 (12.3)	228	36 (15.8)
	Ae. vittatus	21	2 (9.5)	1	0	59	0	133	9 (6.8)	214	11 (5.1)
Jaipur	*Ae. aegypti*	62	11(17.7)	–	–	84	7 (8.3)	78	12 (15.4)	224	30 (13.4)
	Ae. albopictus	–	–	–	–	–	–	16	3 (18.8)	16	3 (18.8)
	Ae. vittatus	1	0	–		1	0	83	17 (20.5)	85	17 (20)
Kota	*Ae. aegypti*	95	5 (5.3)	20	4 (20)	22	4 (18.2)	148	7 (4.7)	285	20 (7)
	Ae. albopictus	–	–	6	1 (16.7)	1	0	–	–	7	1 (14.3)
	Ae. vittatus	–	–	–	–	–	–	84	4 (4.8)	84	4 (4.8)
Total	*Ae. aegypti*	224	25 (11.2)	104	4 (3.8)	290	40 (13.8)	359	40 (11.1)	977	109 (11.2)
	Ae. albopictus	43	9 (20.9)	21	1 (4.8)	65	14 (21.5)	122	16 (13.1)	251	40 (15.9)
	Ae. vittatus	22	2 (9)	1	0	60	0	300	30 (10)	383	32 (8.4)

mission of disease. Although transovarial transmission of dengue has been reported by many earlier workers[3–5] yet its application as the surveillance factor has not been made except one published report[6]. In the present paper we have made a comprehensive study on seasonal and area distribution of transovarially transmitted dengue virus through available *Aedes* species. The observations in the present study suggest that during winter season which is not the active transmission season of dengue in Rajasthan, India[7–8], *Ae. albopictus* has shown maximum percentage of vertically transmitted virus. Our observation to the earlier studies[9] that how *Ae. albopictus* is the reservoir species of virus during inter-epidemic period of dengue. Since the data pertaining to *Ae. albopictus* represent virus isolations from tree hole breeding mosquitoes, present observations on *Ae. albopictus* and its role as reservoir species also highlight tree holes as the crucial etiological foci of virus retention of dengue in Rajasthan, India. Another important lead emerging through present study is the high mosquito infectivity of *Ae. aegypti* during summer and rainy seasons especially from desert districts, Jodhpur and Jaipur. This observation suggests that in Rajasthan, India, owing to tendency of overstorage of domestic water by the inhabitants, mosquito and vertically transmitted virus get pronounced during the summer season which could precede the active transmission season of dengue during following rainy season. The observations reported here may also provide a possible explanation why Rajasthan is the only area in India from where summer epidemics of dengue have been reported[10].

Present paper marks first report on seasonal and area distribution of transovarial transmission of dengue virus in Rajasthan, India. The observations could be very useful in understanding seasonal and area specific emergence of dengue in this region.

Acknowledgement

The authors are grateful to the Department of Science and Technology, Government of India for providing financial support to the present study.

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Received: 24 October 2007 *Accepted:* 31 December 2007