

## Entomological studies for surveillance and prevention of dengue in arid and semi-arid districts of Rajasthan, India

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### Abstract

**Background & objectives:** Rajasthan is one of the dengue endemic states of India. Very few studies have been published on entomological aspects of dengue in this state. Owing to water scarcity, inhabitants in desert areas overstore domestic water which leads to the persistence of dengue vectors within the domestic premises. Area specific knowledge on breeding, key containers and seasonal rhythms of vector population is essential for preparing an effective prevention plan against dengue. Present paper reports results of entomological investigations on dengue vectors in arid and semi-arid districts of Rajasthan.

**Methods:** Longitudinal studies were undertaken during 2004–06 in one arid and two semi-arid dengue endemic districts of Rajasthan. Adult and larval *Aedes* were collected from the randomly selected houses in representative towns and villages with associated details of container types and water storage practices of inhabitants.

**Results:** In urban areas during all the seasons adult house index (AHI) of *Aedes aegypti* was maximum in desert zone (25) and least in semi-arid area with saline river III (1). The difference of AHI during three seasons was statistically significant ( $\chi^2 = 16.1$ ,  $p < 0.01$  for urban; and  $\chi^2 = 50.71$ ,  $p < 0.001$  for rural). Breeding of *Ae. aegypti* among urban settings was maximum in desert zone. During all the seasons cement tanks were the key breeding habitats for *Ae. aegypti* in desert as well as semi-arid areas.

**Interpretation & conclusion:** Water storage habits during summer season emerged to be the risk factor of vector abundance in urban areas of arid and semi-arid settings. A carefully designed study of key containers targeting cement tanks as the primary habitats of mosquito control may lead to commendable results for dengue prevention.

**Key words** *Aedes aegypti* – dengue – India – Rajasthan – surveillance design

### Introduction

Dengue fever (DF) associated with dengue haemorrhagic fever (DHF) is an emerging public health problem in many countries of Asia and South America. As per the recent WHO estimates, dengue is emerging at the rate of 50 million new infections per year in almost 100 disease en-

demic countries of the world including India<sup>1</sup>. In Rajasthan state, India, many outbreaks of dengue have been reported in the past<sup>2–5</sup>. However, the reports published so far pertain to the clinical and virological aspects of dengue outbreaks. Except one report<sup>6</sup> no subsequent studies have been undertaken so far on entomological aspects of dengue in Rajasthan.

Since dengue is transmitted by domestic breeding *Aedes* (Diptera: Culicidae) mosquitoes, and the virus is reported to be maintained through vertical transmission<sup>7-9</sup>, vectors play the dual role of disease transmission during epidemics and virus retention during inter-epidemics period. This etiological aspect of dengue necessitates strengthening of entomological knowledge on dengue in its different socioecological paradigms to develop an effective surveillance and prevention plan against the disease. Rajasthan representing arid, semi-arid and non-arid zones, presents wide heterogeneity in terms of water storage practices. Since transmission of dengue in a household is governed by the number and type of such water-filled indoor utensils, we need to generate entomological knowledge of dengue vectors. With the above objectives, present paper reports the results of longitudinal studies in dengue endemic districts of Rajasthan, carried out from 2004–06.

### Material & Methods

*Selection criteria of the study areas:* Based on the climate, topography and rainfall pattern of areas and sociocultural habits of the inhabitants, the Rajasthan state, is characterized by distinct physiographic regions (Fig. 1). Since the existing stratification carries ecosociological basis which is relevant in studying entomological situation of area for domestic breeding of *Aedes* mosquitoes, three arid and semi-arid physiographic regions, namely Marusthali (Desert zone), Shekhawati (Desert with Aravali ranges), and Luni Basin (Saline river zone) were chosen for the present study.

*Study settings, sampling and periodicity of investigations:* From each of above three zones, four villages and one urban town were selected for the present study. In all, 12 villages and three towns were surveyed periodically during summer, rainy and winter seasons. Within each season, two-point study (first study and first follow-up) had been undertaken, hence data presented here represent an average of two in-

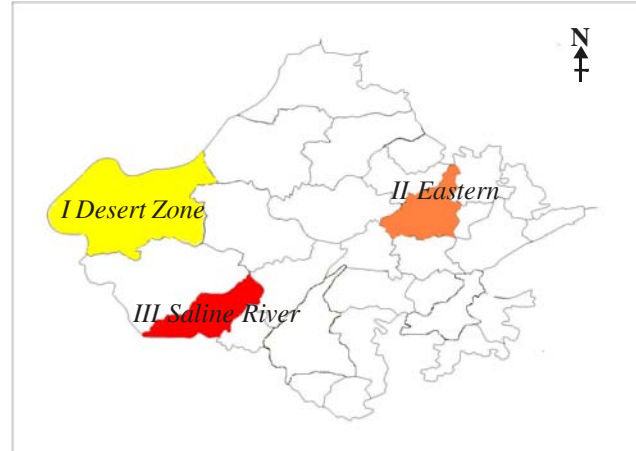


Fig. 1: Map of Rajasthan state showing study areas

vestigations made in each of 12 study villages and three towns during all the three seasons. A cluster of 100 houses or representative sample of available houses selected by systematic random sampling was screened in all the study settings.

*Description of study areas:* The Rajasthan state represents northwestern border of India. The area is situated between 23°3' to 30°12'N longitude and 69°30' to 78°17'E latitude. The entire region is known for its rich cultural heritage. Broadly, entire state, except its southern part, represents an arid or semi-arid environment. However, owing to different topographical and geographical situations, degree of desertification, economy and cultural practices of different areas are different. The three study areas which cover representation of arid and semi-arid ecology are described below:

(i) *Area-I (Desert— Marusthali zone):* This zone is represented by Jaisalmer, Barmer districts and part of Jodhpur district. Jaisalmer district has been selected for the present study which lies between 28°05' to 25°45'N longitude and 69°30' to 73°04'E latitude. This represents true desert. The temperature ranges between 4 and 49°C during the year. The annual rainfall is about 300–360 mm per annum and ambient relative humidity is lowest in the country. The economy of the people is based on rearing of live-

stock such as sheep and goats. The area is frequently challenged by droughts and as a result, native population (except ladies and children <10 years) migrate to neighbouring states for their livelihood and grazing livestock, during summer season.

The average density of human population in the area is 76/km<sup>2</sup>. In this region, due to scarce and scanty water availability/supply, inhabitants used to store water in large number of domestic containers such as cement tanks, underground tanks, clay utensils, metallic tanks, etc. As a water conservation measure, during summer season the domestic containers are never emptied.

(ii) *Area-II (Semi arid— Shekhawati zone)*: This zone is represented by Sikar, Jaipur and Bharatpur districts and extends from northeast to eastern part of the state. Jaipur district has been selected for the present study which lies between 27°37' to 26°03' N longitude and 74°58' to 76°31' E latitude. Northern part of the region represents extreme desert whereas eastern part lies close to the Aravali Hills bordering western side of the zone. The economy of desert part of this region again like desert zone, depends on livestock whereas eastern part of Aravali zone has better ground water level, resulting to relatively easier availability of domestic water, hence less storing habits, at least in rural areas of the zone. The average population density of these districts is about 398/km<sup>2</sup>; however, Jaipur district of this study area is the most populated in the state with a population density of 471/km<sup>2</sup>.

(iii) *Area-III (Semi-desert— Saline river zone)*: This area is represented by part of Jodhpur district, Pali and Jalore districts. Jalore district has been selected for the present study which lies between 25°57' to 24°22' N longitude and 72°10' to 73°38' E latitude. The entire area has main and tributaries of saline River Luni. The weather, rainfall pattern and culture and practices of inhabitants of this area are almost same as that of the desert zone. The average human density of this zone is approximately 136/km<sup>2</sup>.

*Adult and larval mosquito collections*: Adult *Aedes* mosquitoes were collected from sampled human dwellings during daytime using a suction tube and torch. The mosquitoes captured were transferred to Barraud cages wrapped with the wet cloth on 2–3 sides. In the field the mosquitoes were fed on cotton pads soaked with 4% glucose solution. Live mosquitoes were brought to the laboratory for identification using relevant literature<sup>10</sup>. Area-wise pools of adult mosquitoes were maintained in the insectary having temperature of 20–25°C and relative humidity of 70–80%.

Larvae and pupae were collected from all the domestic water containers available in the sampled houses. A container was recorded as positive for breeding when at least one larva was seen in it. All the larvae and pupae collected were maintained in separate glass jars for each of study village and town. In laboratory, sterilized dog biscuits and yeast powder was provided as the larval food. All the larvae collected were transformed into adults and were identified for their species. Adult house index (AHI) was computed as percentage of the houses having adult *Ae. aegypti*, breeding index as percentage of houses having breeding and container indices were computed as percentage of containers showing breeding of *Ae. aegypti*.

The data collected from 15 settings were analyzed using appropriate statistical methods. The paradigm displays showing relationship of observations of entomological parameters and attributes of the area were incorporated in geographical information system (GIS), (Arch view, developed by ESRI, U.S.A.).

## Results

*Adult mosquito collections in urban areas*: Investigations were undertaken in 300 houses of three zones in summer, 300 in rainy and 407 houses during winter season of the year. Only one species (*Ae. aegypti*) was observed in all the domestic premises surveyed. AHI was computed based on overall collection of

1713 adult *Ae. aegypti*. Table 1 shows the data pertaining to variation of AHI of *Ae. aegypti* in three study zones across three seasons. During summer season, maximum AHI was observed in Area I (24) followed by Area II (15) and Area III (6). During rainy season also, maximum AHI was observed in Area I (20) followed by Area II (3) and Area III (1). Inter-regional difference of AHI was found statistically significant. During winter-spring season also urban AHI was highest (25) in Area I followed by 23.3 in Area II and 17.6 in Area III (Table 1). Data indicate that among urban settings of all study areas during all the seasons, Area I (Desert zone) had highest number of houses positive for the presence of adult *Ae. aegypti*.

*Mosquito larval collections in urban areas:* To study the breeding of *Aedes* mosquitoes about 10,981 domestic water containers among urban settings of three study zones during three seasons had been screened, which included 6398 containers in summer, 2490 in rainy season and 2093 containers in winter-spring season. Percentage of containers found positive for breeding has been recorded as breeding index (BI). During summer season BI was maximum in Area I (37), followed by Area II (15) and Area III (5). But during rainy season, BI in Area I was only 5, 14 in Area II, and 4 in Area III. During winter-spring season also, Area I showed only 12 BI as against 48.3 in Area II and 43.3 in Area III (Table 1).

*Adult mosquito collections in rural areas:* Among rural settings of three study areas, 688 houses were searched in summer season, 1188 in rainy season and 1673 houses in winter-spring season for the presence of adult *Aedes*. No adult *Ae. aegypti* was found in rural areas of Area I during summer season. In Area II 12.5 and Area III, 9 AHI was observed. During rainy season also, no adult *Ae. aegypti* was found in Area I. However, in Area II 14.5 and in Area III 5.5 AHI was observed. In winter-spring season also no adult *Aedes* were observed in Area I, whereas in Area II 7.3 and in Area III 6.8 AHI was recorded (Table 1).

*Mosquito larval collections in rural areas:* We examined 18,489 domestic containers for the presence of larval *Aedes* during summer season, 6967 during rainy season and 8233 containers during winter-spring season in rural settings. Among rural settings during summer season, BI observed in Area I was 60, in Area II, 94 and Area III 19. Maximum rural BI (94) was observed in Area II, followed by Area I (60). In rural areas during rainy season, in Area I, 2.2 BI in Area II 23 and in Area III 9.7 BI was observed (Table 1). During winter-spring season, among rural areas 0.05% containers were found breeding positive in Area I, 4.43% in Area II and 1.16% in Area III (Table 1). Maximum BI (25.7) was observed in Area II.

*Breeding habitats and key containers:* In study area

**Table 1. Inter-regional, inter-seasonal prevalence of adult and larval *Aedes aegypti* in three study zones**

Area		Summer		Rainy		Winter-spring	
		AHI	BI	AHI	BI	AHI	BI
Area I	Urban	24 (100)	37 (1763)	20 (100)	5 (395)	25 (100)	12 (428)
	Rural	0 (288)	60 (5731)	0 (388)	2.6 (1335)	0 (594)	0.16 (1942)
Area II	Urban	15 (100)	15 (1907)	3 (100)	14 (831)	23.3 (120)	48.3 (564)
	Rural	12.5 (200)	94 (5538)	14.5 (400)	23 (2453)	7.3 (684)	25.7 (3965)
Area III	Urban	6 (100)	5 (2728)	1 (100)	4 (1264)	17.6 (187)	43.3 (1101)
	Rural	9 (200)	19 (7220)	5.5 (400)	9.7 (3179)	6.8 (395)	6.8 (2326)

AHI-Adult house index; BI- Breeding index; Figures in parentheses are number of houses searched/containers examined.

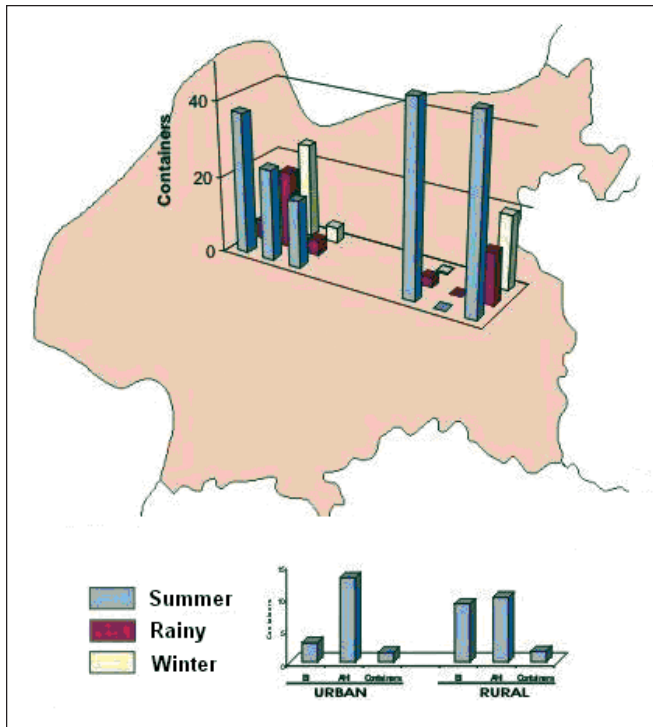


Fig. 2: Inter-seasonal comparison of adult and larval *Aedes* breeding in Area I

I, among urban settings during summer season, AHI was 24 whereas corresponding BI was 37. In rural areas, AHI was nil whereas, BI was 60. The association of AHI with BI in urban areas highlights favourable conditions for breeding as well as adult survival of *Aedes* during summer season, whereas in rural areas it indicates less protected domestic habitats for the successful survival of adult *Aedes* (Fig. 2 blue pillars). A resolution of different breeding habitats of *Aedes* in urban and rural settings during summer season is depicted in Table 2. Maximum container index (12.9%) has been observed in 147 cement tanks followed by 5.8% of 204 underground water storage tanks and 5.5% of 54 metal tanks examined. On the other hand, in rural areas during summer season, 9.3% of cement tanks examined ( $n = 224$ ) were positive for breeding followed by 4% of 691 underground tanks and only 0.5% of 383 metallic tanks (Table 2). A positive association of number of containers with breeding index is shown in Fig. 2. In urban settings, out of 1763 breeding sites exam-

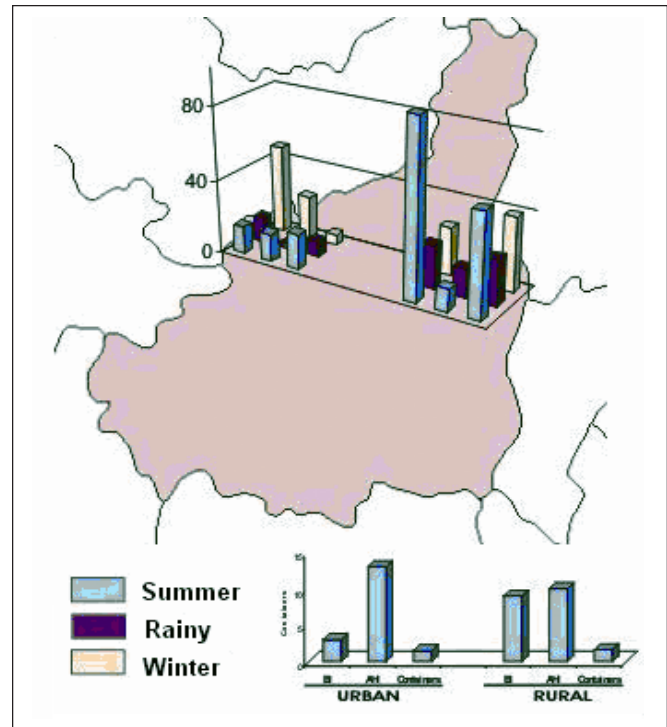


Fig. 3: Inter-seasonal comparison of adult and larval *Aedes* breeding in Area II

ined, 6% were breeding positive, whereas in rural settings, of 5731 containers examined only 2.1% positive (Fig. 2). The data reflect that cement and underground tanks were the preferred breeding sites of *Aedes* mosquitoes in summer season in desert, whereas metallic, clay and plastic tanks as least preferred breeding habitats (Table 2).

In study area II, AHI in urban (15) and rural (12.5) settings were almost similar. BI in urban areas was 15% whereas, in rural areas it was 94% (Fig. 3). More breeding among rural setting is an observation common with areas I and II. A resolution of different breeding habitats of urban and rural areas of Area II during summer season is depicted in Table 2. Among urban areas, the maximum container index (41.6%) has been observed in 12 other containers examined, followed by coolers (14%) and cement tanks (7.9%). water filled metallic tanks, clay pots and plastic containers were the least preferred sites of breeding (Table 2).



**Table 2. Percent positivity of containers examined for the breeding of *Aedes aegypti* during different season**

Area	Type of containers							Total	
	Cement	Metallic	Clay	Plastic	Underground tanks	Coolers	Others		
<i>Summer</i>									
Area I	Urban	12.9 (147)	5.5 (54)	0 (1184)	2.9 (174)	5.9 (204)	0 (0)	0 (0)	6.1 (1763)
	Rural	9.3 (224)	0.5 (383)	1.7 (3960)	1.3 (473)	4 (691)	0 (0)	0 (0)	2.1 (5731)
Area II	Urban	7.9 (429)	1 (384)	0.5 (639)	0 (287)	4.3 (92)	14 (64)	41.6 (12)	2.1 (1907)
	Rural	23.3 (1114)	0.3 (1149)	0.6 (2415)	0 (610)	6.2 (176)	20.3 (54)	50 (20)	3.9 (5538)
Area III	Urban	4.8 (313)	0.4 (275)	0.1 (1490)	0 (346)	4 (175)	0 (27)	3.9 (102)	1 (2728)
	Rural	9.3 (920)	2.3 (1013)	1.2 (4255)	0 (343)	1.4 (551)	48.1 (27)	5.4 (93)	2.6 (7220)
<i>Rainy</i>									
Area I	Urban	11.1 (27)	4.5 (22)	1.6 (250)	2 (48)	0 (48)	0 (0)	0 (0)	2.3 (395)
	Rural	1.9 (52)	0 (163)	0.2 (846)	0 (125)	1.3 (149)	0 (0)	0 (0)	0.3 (1335)
Area II	Urban	8.5 (187)	2.2 (174)	0.4 (271)	0 (133)	0 (40)	12.5 (24)	50 (2)	3 (831)
	Rural	21.9 (56)	0.6 (499)	0.5 (1047)	0 (269)	8.8 (68)	20.4 (54)	20 (10)	5.6 (2453)
Area III	Urban	6.12 (147)	0.8 (125)	0.1 (692)	0 (160)	3.6 (83)	0 (11)	8.6 (46)	1.4 (1264)
	Rural	12.8 (420)	2.3 (477)	1.9 (1885)	0 (167)	2.9 (203)	20 (5)	4.5 (22)	3.4 (3179)
<i>Winter-spring</i>									
Area I	Urban	16.2 (37)	0 (48)	1.7 (229)	2.8 (71)	0 (43)	0 (0)	0 (0)	2.8 (428)
	Rural	1.3 (77)	0 (272)	0 (1098)	0 (196)	0 (299)	0 (0)	0 (0)	0.1 (1942)
Area II	Urban	34.2 (146)	1.7 (118)	0.5 (186)	1.2 (86)	11.5 (26)	0 (0)	50 (2)	10.3 (564)
	Rural	27.9 (580)	0.3 (718)	0.2 (1947)	0.2 (577)	4.9 (143)	0 (0)	0 (0)	4.4 (3965)
Area III	Urban	8.8 (215)	6.2 (129)	8.7 (531)	5.4 (129)	0 (88)	11.1 (9)	0 (0)	7.3 (1101)
	Rural	4.9 (350)	0.2 (392)	0.5 (1265)	0.9 (105)	0.5 (190)	0 (2)	0 (22)	1.2 (2326)

Figures in parentheses are total number of containers examined.

In study area III, AHI in urban areas was only 6% whereas in rural areas it was 9%. This reverse trend of more AHI in rural than urban areas was different as compared to the other two areas where more AHI was observed in urban set ups than rural ones. But the breeding profile in this area appeared similar to that of other two areas—more in rural set ups (19%) than urban (5%) (Fig. 4). A resolution of different breeding habitats of urban and rural areas during summer season is depicted in Table 2. In urban areas of Area III (Saline river zone), the maximum container index has been observed in 313 cement tanks examined (4.8%), followed by underground tanks (4%). A similar trend of least breeding in clay, metallic and plas-

tic containers as observed for Areas I, II, was also observed among urban settings of this area. In rural areas of this zone, maximum breeding (48.1%) was observed in 27 coolers followed by cement tanks (9.3%). Like other areas, here also water filled metallic tanks, clay and plastic containers were the least preferred breeding habitats (Table 2).

### Discussion

Study of variation of AHI in urban settings revealing that Desert zone (Area I) and semi-arid area (Area II) represent maximum AHI during summer season, explains why desert is the only area in India where

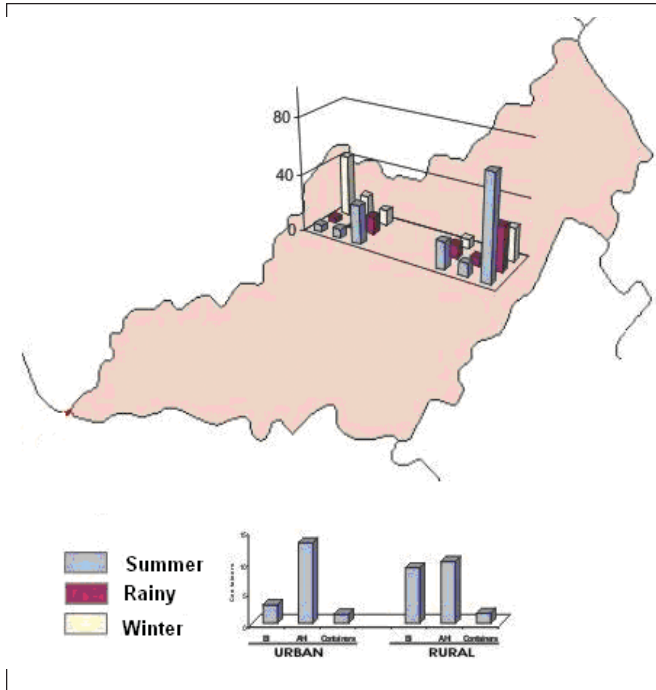


Fig. 4: Inter-seasonal comparison of adult and larval *Aedes* breeding in Area III

epidemics of dengue have been reported during summer season<sup>11</sup>. Association of AHI with more number of containers for domestic use could be due to the reason that owing to arid conditions in this area, people store more and more water in as many domestic containers, thereby providing abundant breeding habitats to *Ae. aegypti*. Based on these observations and on socioeconomic and educational status, the population in towns can easily be stratified into the categories with a particular pattern of storing domestic water. However, limitation of above indicators is that these may be predictive only during summer season when people have more tendency of storing water and least attempts to empty their containers. Association of number of containers, BI and resultant AHI represent a linear relationship. Understanding this relationship number of domestic containers during summer season may serve to predict prospective AHI. However, during other seasons, tangential or indirect factor such as the emptying habits of inhabitants may be influencing this association. There-

fore, for a model development provision for linearly as well as tangentially related factors need to be made.

Like summer season, in urban settings during rainy season also, maximum AHI was observed in Area I followed by Area II. Number of containers were associated with the BI except area III. The reason being simple Area III being representative of desert only, possess less protective conditions for mosquito survival. During rainy season, due to better availability of water, the habits of storing water are not much pronounced and the criteria of number of containers may not be suitable for surveillance plan. Hence, for all the arid and semi-arid settings of Rajasthan state during rainy season, a uniform policy of adult and larval control based on the ecological criteria will be sufficient.

In all the areas except Area II, spring-winter season (December to March) appears to be the most favourable for adult mosquitoes in urban settings. But more AHI and BI in this season were not associated with number of containers, probably due to better survival of adult mosquitoes during March and April. Similar observations have been reported from the Area III in our earlier studies also<sup>12</sup>. Therefore, like urban settings, similar observations could be gathered from rural settings also. In the absence of any association between number of containers and BI, ecological criteria appear to be suitable basis of stratification for dengue surveillance.

Unlike urban areas, rural areas showed uniformity in terms of association of number of containers with breeding indices during summer. Due to sparse housing pattern as compared to their urban settings, rural areas did not appear to differ in their behaviour among all the study regions. A common rural plan based on socioeconomic status can thus be formulated for summer season. In Rajasthan, a three pronged programme of urban dengue vector control during summer needs to be developed. Based on the

observations discussed so far, following surveillance guidelines have emerged: (i) based on socioeconomic criteria in Areas I, II and III and other similar settings in the state a stratification of regions can be developed for surveillance and control of dengue vectors; and (ii) based on socioecological criteria, rural areas of all the three regions of the state can be stratified and surveillance and control of vectors could be taken up.

During rainy season, in rural settings of Area I, no adult mosquitoes could be collected, whereas a relatively much lower BI was observed as compared to other study areas. This could be probably due to the fact that corresponding AHI was nil in these settings and hence a low breeding index was resulted. Since owing to rains number of containers in these settings was much less as compared to the same during summer, the poor presence of adult mosquitoes could not be compensated in rainy season. The observations suggest that even larval control will suffice an effective vector reduction in rural areas during rainy season. In other study areas, namely Area II & III, a uniform pattern of association of more BI with more number of containers was observed.

The results show that cement tanks, coolers and underground tanks are the most preferred breeding habitats in urban areas for all the settings of three study areas in summer season and could be the target points of larval control priorities. The results show that a common programme for rural settings of all study areas may be effective for both the summer and rainy seasons whereas, for urban settings, area-wise plans may be required in summer and rainy season separately. During winter-spring, one common vector control plan applicable to all the areas and the settings should be opted.

There have been a number of studies on entomological aspects of dengue from India<sup>12-16</sup>. However, what has not been conceptualized is the fact that ecological diversities coupled with sociocultural heterogeneity in the country like India, leads to the availability

of many 'dengue vector systems'. Living style of people especially their water storage practices, agglomeration pattern of dwellings and ecology of settings are the significant attributes which when grouped impart the status of an independent dengue system to a population group. Knowledge of such system across the country needs to be derived to develop a region specific entomological surveillance system, as has been attempted in the present study. Once criteria of stratification, seasons of surveillance and key containers in a setting are identified, further attempts, as done by workers in different parts of the world<sup>17-19</sup> may be accomplished in endemic areas also. Only three studies have been reported so far<sup>4,6,12</sup> on entomological aspects of dengue in Rajasthan. Results of the present investigation will demonstrate a working model of situation analysis and risk prediction of dengue vectors in any setting of Rajasthan and could be useful for developing dengue vector control strategy in urban and rural areas of desert and semi-desert conditions in Rajasthan.

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