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Resting behaviour of *Anopheles stephensi* type form to assess its amenability to control malaria through indoor residual spray

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ABSTRACT

Background & objectives: In the present study, an attempt was made to find reasons of ineffectiveness of indoor residual spray to interrupt malaria transmission by investigating the behaviour of two variants of *Anopheles stepnesi,* viz. type form and *mysorensis* in rural areas of Rajasthan, India.

Methods: Both low malarious (DDT spray from 1958 to 1976) and high malarious (DDT spray from 1958 to April 2006) villages were selected for the study in Arid zone district Jodhpur, Rajasthan during March–April 2006. Resting behaviour of the species during all its movement rhythms covering 24 h period related to: (i) swarming/ mating; (ii) pre- and post-biting rest; (iii) after feed resting between hoping movements; (iv) night and day time resting and; (v) diel activity movements in response to temperature changes, were carried out.

Results: The results showed that household objects like cupboards, furnitures, hanging clothes, goods stacked on loft, stored clothes, cobwebs and floor were the dominant resting sites both in sprayed and unsprayed villages. About 95 and 97% of *An. stephensi* preferred to rest on household objects of unsprayed and sprayed villages respectively. There was no significant difference in resting behaviour of the species in both groups of villages ($p > 0.05$). The pre-biting resting time was recorded as 5 to 15 min whereas post-biting resting time lasted for 15 to 25 min. After biting outdoor (in courtyard – open to sky) species starts entering the rooms at around 2330 hrs. It was observed that during III quarter (0100 to 0400 hrs) maximum species entered into the room were 56% in unsprayed and 62% in sprayed villages. Statistically there was no significant difference in the entry of mosquitoes ($p > 0.05$) in both the groups of villages.

Conclusion: Before DDT era, *An. stephensi* was found resting at all heights of the walls inside the human dwellings. Present study revealed that *An. stephensi* is trying to avoid sprayable surfaces and tend to rest on unsprayable surfaces during all its movement rhythms starting from swarming, pre-/post-biting and during hopping movements in the courtyard and thereafter household objects inside the room as final day time resting. It was concluded that changed behaviour of resting of *An. stephensi* on unsprayable objects in sprayed rooms largely accounted for failure of malaria control. Control of *An. stephensi,* thus requires an integrated vector control strategy based upon interesectoral, environmental, larviciding with chemical/biolarvicide and use of larvivorous fish wherever feasible. Such a control strategy offers cost-effective and sustainable option than indoor residual spray.

Key words *Anopheles stephensi;* diel activity; India; indoor resting; pre- and post-biting rests; Rajasthan; sprayed surfaces

INTRODUCTION

Anopheles stephensi essentially a species of Oriental Region is wide spread in eastern mediterranean region in Saudi Arabia, Iraq, Iran, Pakistan and towards east it extends up to India[1]. *An. stephensi* exists as two variants, i.e. type form and *mysorensis* and can be separated on the basis of ova (>14 ridges on one side float – type form and up to 14 in *mysorensis*)[2,3]. Both the variants carry high vectorial capacity. In Asia west of India (Iraq and Iran), *An. stephensi mysorensis* is a most prevalent variant and extensively breeds in open terrain systems, viz. irrigation/seepage channels, marshy/stagnant pools and is responsible for large malaria epidemics[4].

In India, the species is prevalent in dry and hot climate conditions in Punjab, Haryana and Rajasthan and in all urban areas throughout the country. Both the variants have adapted as container habitat species in both rural and urban areas. It breeds in cisterns, overhead tanks, underground cisterns (*tankas*), wells, pots and other water storage receptacles[4].

Attempts were made to identify these two variants on the basis of spiracular indices in rural areas of Rajasthan. In *An. stephensi* type form, average spiracle length was 0.11–0.12 mm and average spiracular index was 8.09–9.23, whereas in *mysorensis*, the corresponding figures were 0.09–0.10 mm and 6.82–7.60[3]. The type form is exclusively domestic in all the seasons, whereas the *mysorensis* variant occupies the outdoor niche during monsoon and post-monsoon seasons, with spillover into domestic sites during summer-ecological stress period[5].

Therefore, the role of *mysorensis* in malaria transmission appears to be restricted to spring season (March–April) only[6]. During monsoon period (July–September) *mysorensis* variant occupies outdoor niches and is totally zoophagic. However, in urban areas throughout the country, both the variants occupy domestic habitats and are involved in the transmission of malaria.

National Malaria Eradication Programme (NMEP) in the country started in 1958 and Rajasthan received two rounds of spray of DDT @ 1 g/m^2 during transmission season. Malaria cases in the country showed decline in 1964 but resurged again during 1976. Rajasthan also had its share of resurgence. To meet the challenge, Modified Plan of Operation was launched in 1976 which aimed at: (i) preventing deaths due to malaria; and (ii) reducing morbidity: vector control strategy included selective DDT spray in villages with >2 API (Annual parasite incidence/1000 population). In District Jodhpur, it was observed that DDT spray in villages with >2 API failed to bring down the incidence of malaria. In order to investigate the reasons for failure of interruption of malaria transmission, the present study was planned during March–April 2006.

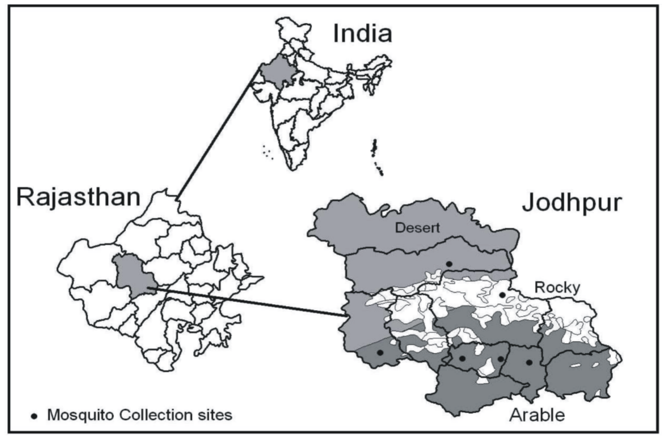

Fig. 1: Location of District Jodhpur and sites for the observation of 24 h movement rhythms of *An. stephensi*.

METHODS

Study area

District Jodhpur situated in eastern part of Thar Desert (Rajasthan, India) lies between 26^o 0" and 27^o 37" north latitude and 72^o 55" and 73^o 52" east longitude. The District receives 30 to 35 cm rainfall annually and the temperature ranges from 15.5 to 42.2^{o}C.

During the study, a set of 4 low malarious villages where IRS is not undertaken by the national programme. (Annual parasite incidence <2) and 2 high malarious villages where IRS is being regularly undertaken by the national programme (Annual parasite incidence >2) spread over different parts of the district were selected for the study (Fig. 1). Low malarious villages did not receive DDT spray as per verifiable records for the last 10 years while high malarious villages received two rounds of DDT spray @ 1 g/m^2 as per schedule of the National Anti Malaria Programme (NAMP) now National Vector Borne Disease Control Programme (NVBDCP).

Housing pattern

Housing pattern is an important element in determining the resting behaviour of mosquitoes. Thar Desert in Rajasthan, is thinly populated as compared to the other regions of Rajasthan. Thar has 'Gypsum' layer (impermeable layer) which does not allow percolation of water. Hence, Thar Desert has more availability of water than other desert regions of the world. Houses in the region are built of stone/brick walls with stone slab/concrete roofs. In view of low human density and easy availability of land structures, an average house comprise of 1 to 3 rooms, verandah plus open courtyard. Low sub-soil water level ensures comparative dryness of these structures.

Plan of work

The observations were made in 18 rooms of 6 villages. In each village, 3 rooms were selected on the basis of abundance of the mosquitoes. The mosquito resting sites inside the rooms included sprayable structures, i.e. walls, roof, windows and unsprayable structures, i.e. household objects. The temperature of the household objects, side walls, and outside the room (ambient) was recorded by sensor thermometer at an hourly interval. Data were recorded from 0700 to 0600 hrs (24 h cycle) indoors on hourly basis. While 1845 hrs onwards the observations were extended to outdoors also, each day, to cover outdoor movements of the mosquitoes. Resting count was made carefully to avoid disturbance and consequent resettling and recount[7]. Actual collections were made only from animal/human baits to assess the biting preferences of the species indoors and outdoors. Landing mosquitoes were collected to prevent biting and also prophylaxis doses were given to the human bait. Before starting the experiment village community was taken into confidence through discussions with the village heads about the work to enlist their whole-hearted support.

Statistical analysis

Z-test was used to confirm the field results on: (i) resting behaviour of *An. stephensi* on sprayed and unsprayed surfaces in both the groups of villages; (ii) preferred resting sites of the species in both the groups of villages; (iii) quarterly entry of species during night after

Table 1. Daytime indoor resting sites of *An. stephensi* in Jodhpur during March–April 2006

	Time (hrs)	Unsprayable surface (Household goods)									Sprayable surfaces (House structures)				Grand total
		Cups boards	Furniture	Hanging clothes	Lofts	Stored clothes	Utensils	Cob-webs	Floors	Total	Windows	Roofs/ Ceilings	Walls	Total	
Unsprayed villages (4 villages)															
Quarter I	0700–1000	293 (17.4)	22 (1.3)	524 (31)	31 (1.8)	138 (8.2)	524 (31)	136 (8.1)	20 (1.2)	1688	2 (4.3)	3 (6.4)	42 (89.4)	47	1735
Quarter II	1000–1300	266 (16.1)	23 (1.4)	562 (34)	29 (1.8)	151 (9.1)	485 (29.3)	120 (7.3)	19 (1.1)	1655	1 (3)	1 (3)	31 (93.9)	33	1688
Quarter III	1300–1600	297 (17.8)	30 (1.8)	550 (33)	45 (2.7)	138 (8.3)	483 (28.9)	111 (6.7)	15 (0.9)	1669	0 (0)	0 (0)	27 (100)	27	1696
Quarter IV	1600–1845	202 (15)	26 (1.9)	465 (34.4)	45 (3.3)	100 (7.4)	409 (30.3)	91 (6.7)	13 (1)	1351	0 (0)	0 (0)	21 (100)	21	1372
	1845–1900	9 (13.4)	2 (3)	32 (47.8)	3 (4.5)	5 (7.5)	14 (20.9)	2 (3)	0 (0)	67	0 (0)	0 (0)	2 (100)	2	69
Sprayed villages (2 villages)															
Quarter I	1900–1000	166 (23.6)	15 (2.1)	189 (26.9)	13 (1.9)	66 (9.4)	187 (26.6)	49 (7)	17 (2.4)	702	1 (2.7)	2 (5.4)	34 (91.9)	37	739
Quarter II	1000–1300	134 (18.6)	17 (2.4)	193 (26.8)	20 (2.8)	81 (11.2)	216 (30)	44 (6.1)	16 (2.2)	721	0 (0)	0 (0)	22 (100)	22	743
Quarter III	1300–1600	137 (18.9)	16 (2.2)	197 (27.1)	36 (5)	63 (8.7)	242 (33.3)	18 (2.5)	17 (2.3)	726	0 (0)	0 (0)	23 (100)	23	749
Quarter IV	1600–1845	159 (22.9)	10 (1.4)	205 (29.5)	33 (4.7)	74 (10.6)	174 (25)	26 (3.7)	14 (2)	695	0 (0)	0 (0)	20 (100)	20	715
	1845–1900	6 (9.8)	0 (0)	45 (73.8)	2 (3.3)	2 (3.3)	5 (8.2)	1 (1.6)	0 (0)	61	0 (0)	0 (0)	1 (100)	1	62

Figures in parentheses indicate percentages.

the feeding in sprayed and unsprayed rooms; and (iv) impact of temperature on the diel activity of the species.

RESULTS

Daytime resting sites

Anopheles stephensi (>90% of the total collection) was the dominant species in both the sets of villages. Results of day-time indoor resting count in the sprayable and unsprayable surfaces in both the groups of villages are given in Table 1. From the data it is apparent that in unsprayed villages, household objects (>97%), viz. cupboards, furnitures, hanging clothes, goods stacked on lofts, stored clothes, cobwebs and floor were the dominant resting sites. Sprayable surfaces, viz. windows, roofs/ceilings and walls accounted for only 3% collection. In sprayed villages, these figures were 95 and 5% respectively. There was no significant difference in both the groups of villages in the resting behaviour on unsprayed surfaces (p >0.05). Among household objects in both the groups of villages, hanging clothes, utensils followed by cupboards were the most preferred resting sites while among sprayable surfaces, walls were the preferred sites. There was no significant difference on the preferred resting sites (p >0.05) in both the groups of villages.

Swarming/Mating

Anopheles stephensi (except semi-gravid) females were observed to start leaving the daytime resting sites (premises) by 1830 hrs for swarming/feeding/oviposition. Swarming and mating was observed to start around 1845 hrs in the courtyard on different objects at 2–3.5 m height and lasted for 15 to 20 min.

Pre- and post-biting rest

Biting starts immediately after swarming by 1915 hrs. The maximum biting was recorded in courtyards as compared to indoors. Before and after biting it was observed that the species preferred to rest on unsprayable surfaces in the courtyards. The pre-biting resting time was recorded as 5 to 15 min whereas post-biting resting time lasted for 15 to 25 min.

Hopping movement after post-feeding

Fed mosquitoes hopped to different unsprayable sites for temporary rest in the courtyards only, till the temperature inside rooms fell down to 29 to 30^{o}C. Around 2330 hrs the mosquitoes start entering the rooms for final rest and this process is completed by sunrise.

Room entry by nighttime/quarterly entry of mosquitoes

Nighttime resting observations with respect to both the groups of villages are included in Table 2. It may be observed that out of total fed mosquitoes which entered the rooms on unsprayable surfaces, 13, 56 and 30% ac-

Table 2. Nighttime indoor resting count of fed females of *An. stephensi* in Jodhpur during March–April 2006

	Time (hrs)	Unsprayable surface (Household goods)					Sprayable surface (House structures)	Grand total
		Hanging clothes	Stored clothes	Utensils	Furnitures	Total	Walls	
Unsprayed villages (4 villages)								
Quarter I	1900–2330	Swarming/mating pre-biting, post-biting and resting connected with hopping flights in the open courtyard						
Quarter II	2330–0100	25 (49)	10 (19.6)	15 (29.4)	1 (2)	51	1 (100)	52
Quarter IIII	0100–0400	147 (54.4)	37 (13.7)	81 (30)	5 (1.9)	270	4 (100)	274
Quarter IV	0400–0700	180 (46)	49 (12.5)	154 (39.4)	8 (2)	391	6 (100)	397
Sprayed villages (2 villages)								
Quarter I	1900–2330	Swarming/mating pre-biting, post-biting and resting connected with hopping flights in the open courtyard						
Quarter II	2330–0100	15 (48.4)	5 (16.1)	10 (32.3)	1 (3.2)	31	1 (100)	32
Quarter IIII	0100–0400	98 (47.8)	35 (17.1)	66 (32.2)	6 (2.9)	205	5 (100)	210
Quarter IV	0400–0700	143 (51.3)	39 (14)	91 (32.6)	6 (2.2)	279	6 (100)	285

Figures in parentheses are percentages.

Table 3. Day temperature of objects, walls and outside the rooms (ambient) in the study area during March–April 2006

Quarter	Time (hrs)	Average temperature (°C) (minimum to maximum)		Ambient temperature (°C) minimum to maximum outside the rooms
		Objects	Walls	
Quarter 1	0700–1000	27.5–30.4	28.5–34.42	29.9–35.6
Quarter II	1000–1300	31.5–33.5	35.1–37.4	35.9–39.54
Quarter III	1300–1600	34.0–35.02	36.8–38.9	39.3–40.8
Quarter IV	1600–1900	35.0–35.5	37.5–40.2	40.8–41.4

counted for II, III and IV quarters respectively in the night in unsprayed villages, whereas 11.2, 62.4 and 26% respectively in sprayed villages. Statistically, there was no significant difference in the entry of mosquitoes in different quarters in both the groups of villages (p >0.05). Nearly, 98% of mosquitoes selected household objects as resting sites in both the groups of villages. Statistically there was no significant difference in resting of mosquitoes on the unsprayed surfaces (p >0.05). Hanging objects and utensils were the preferred sites among household objects in both the groups of villages.

Diel activity

Quarter-wise daytime minimum and maximum temperatures with respect to hanging objects, walls and outdoors (ambient) are given in Table 3. Minimum temperature on household objects ranged from 27.5°C in the I quarter to 35°C by IV quarter (rise of 7.5°C) whereas maximum temperature ranged from 30.4 to 35.5°C respectively showing a rise of 5°C. Minimum temperature on walls ranged from 28.5°C during I quarter to 37.5°C in the IV quarter showing a rise of 9°C whereas maximum temperature ranged from 34.4 to 40.6°C (rise of 6.2°C). Higher temperature of walls was due to direct exposure of external side of walls to sunlight. Ambient minimum temperature of 29.9°C and maximum temperature 41.4°C was recorded in I quarter and in IV quarter respectively.

Analysis by quarters did not depict any significant variations in resting behaviour in both the groups of villages with respect to unsprayable and sprayable surfaces. However, diel activity studies on walls, showed significant movement (Table 1). It appears that mosquitoes start leaving the walls (resting sites) as the temperature crosses the level of 35°C. By the IV quarter reduction in the unsprayed group of villages was 50% whereas in sprayed villages it was 42% in response to increasing temperature of the wall. There was no significant difference in the reduction on the number of mosquitoes on the wall due to increase in temperature (p >0.05). Similar response to rising temperature was made in Thane district in respect of three species, viz. *An. culicifacies*, *An. fluviatilis* and *An. stephensi* var. *mysorensis*[8].

DISCUSSION

Jodhpur district has been under DDT spray since the inception of NMEP from 1958 onwards. Selection pressure of DDT is quite high and recent susceptibility studies carried out in the district revealed high degree of resistance as the mortality recorded at 4% discriminating dose ranged from 0 to 14%[9]. It appears that the selection pressure over the last 5 decades has resulted in development of resistance to DDT all over the area.

Before DDT era, *An. stephensi* was found resting throughout the height of walls inside the human dwellings in western India and Punjab now in Pakistan[10]. Present studies have brought that besides physiological resistance, *An. stephensi* has changed its resting behaviour to avoid sprayable surfaces. This selection is so intense that species tend to rest on unsprayable surfaces during all its movement rhythms starting from swarming, pre-/post-biting and during hopping movements in the courtyard and thereafter household objects inside the rooms as final daytime resting.

Anopheles stephensi over the range of its distribution starting in Middle East depicted the same behaviour. Failure of interruption of transmission by DDT as indoor residual spray in southern Iraq during 1968 in spite of > 84% household coverage was attributed to physiological resistance and avoidance of sprayed surfaces as resting sites[11]. In Iran during 1960s, when dieldrin was replaced to control DDT resistant population of *An. stephensi*, the species survived the high toxicity of the insecticide through exophily in the Zagros mountain areas[12]. Behaviour of *An. stephensi* as observed in Jodhpur is in conformity with the behaviour of the species over the range of its distribution. As in Sathenur Dam in

Karnataka where *An. culicifacies* got selected for exophily and exophagy and maintained extra-domiciliary transmission due to the excito-repellency of insecticide, whereas in Jodhpur, Rajasthan *An. stephensi* adapted the resting behaviour change indoor, i.e. to rest on unsprayable surfaces[13].

Persistence of transmission in DDT sprayed areas in the study area can be attributed to physiological resistance and/or avoidance of sprayable surfaces coupled with extended transmission period, i.e. March to October whereas IRS under NAMP covered only May–October period. Farid stressed the importance of the role of Research Centers in the dissemination of new knowledge to programme managers/public heath authorities to enable them to modify/develop new strategies for the control of malaria vectors[14].

Therefore, an integrated control strategy based upon intersectoral approaches, environmental, bednet (LLIN), larviciding with chemical/biolarvicide, and use of larvivorous fish, wherever applicable will be most effective and sustainable option to control *An. stephensi* transmitted malaria in the study area. From the cost-effective and sustainability point of view it would be desirable to eliminate/treat few domestic and peri-domestic breeding sites rather than spray large surface areas of the houses.

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