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Insecticide susceptibility of *Phlebotomus argentipes* & assessment of vector control in two districts of West Bengal, India

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***Background & objectives*: Kala-azar or visceral leishmanisis (VL) is known to be endemic in several States of India including West Bengal (WB). Only meager information is available on the vector dynamics of its vector species, *Phlebotomus argentipes* particularly in relation to control measure from this State. Hence, a pilot study was undertaken to assess the control strategy and its impact on vector in two endemic districts of WB, India.**

***Methods*: Two villages each from the two districts, Maldah and Burdwan, were selected for the study. Seasonal variation of sandflies was observed during pre-monsoon, post-monsoon and winter seasons. Susceptibility test of *P. argentipes* against DDT and bioassay on DDT sprayed wall and on long lasting insecticide nets (LN) PermaNet® 2.0 were conducted as per the WHO standard methods.**

***Results*: *P. argentipes* density was high during March to October. Susceptibility status of *P. argentipes* ranged from 40 to 61.54 per cent. Bioassay test showed 57.89 per cent mortality against LN PermaNet®-2.0. and 50 per cent against DDT on wall within 30 min of exposure.**

***Interpretation & conclusions*: Despite the integrated vector management approach, the sandfly population was high in the study area. The reason could be development of resistance in *P. argentipes* against DDT and low effectiveness of LN PermaNet®-2.0. The more pragmatic step will be to conduct large studies to monitor the susceptibility level in *P. argentipes* against DDT.**

Key words Bio-assay - breading habitat - *Phlebotomus argentipes* - susceptibility - visceral leishmaniasis

Kala-azar or visceral leishmaniasis (VL) is caused by protozoan parasites of the genus *Leishmania,* transmitted to a human host by the bite of an infected female sandfly *Phlebotomus argentipes* (Diptera-Psychodidae)[1,2]. The disease has been reported from 109 districts (Bangladesh 45, India 52 and Nepal 12)[3]. The success of previous vector control efforts was mainly related to the fight against malaria with 4 per cent DDT but the situation was further aggregated by the development of tolerance in *P. argentipes* against DDT[4]. However, within a few years after the end of the eradication efforts, VL returned to Bihar and West Bengal States in India and to Bangladesh[5]. Earlier reports[6-11] pointed towards the development of resistance

in *P. argentipes* against DDT. However, the selection of insecticide is imperative as propensity of sandflies is expected to fluctuate from one region to another depending on its history of spraying and frequency of spray[12-14]. In India, VL is a serious problem in Bihar, Jharkhand, West Bengal and eastern Uttar Pradesh[15]. The levels of sandfly density and allocation of sandfly propensity to insecticides have not been considered in the endemic sites of West Bengal (unpublished observation). Therefore, to develop control measures against sandfly populations in relation to VL and to provide a cogent outline for selecting the suitable insecticide the present study was carried out in the two VL endemic districts, Maldah and Burdwan of West Bengal, India.

Material & Methods

The study was conducted in two villages each in Maldah and Burdwan districts. The villages were selected based on the last three years report of kala-azar cases collected from the public health centres (PHCs). Name of the villages in Maldah were Tapsahor (Habibpur, PHC), and Nimua (Muchia PHC). Similarly, in Burdwan district villages Barapolason tola (Barapolason PHC) and Mahishpur (Bitra PHC) were selected. In these districts, the average temperature in hot season remains about 32°C and at 19°C in the cold season. The annual average rainfall is 150 mm. The maximum precipitation occurs during the period from June to September. Relative humidity ranges from 70 to 90 per cent.

In Maldah and Burdwan districts for the control of kala-azar two rounds (April-May and November-December, 2010) of DDT spray were done and simultaneously long lasting insecticidal nets (LN) PermaNet® 2.0 [purchased from Vestergaard Frandsen, (India) Pvt. Ltd, New Delhi, India] were provided to the households in the endemic villages.

Sandfly collection and identification: From the month of March 2011 to December 2012 Phelotomine and *Sergentomyia* sandflies were collected from both inside (*e.g*. human dwellings and cattleshed) as well as outside (adjacent peridomestic vegetated areas and abandoned houses). Collection was done with CDC light trap (John W. Hock Company, USA) and the density was estimated per trap per night[16]. Traps were installed randomly in selected habitat *i.e.* human dwelling, mixed dwelling (habitat for both human and livestock) and adjacent vegetation. The collected sandflies were sorted, identified, differentiated into different species by examining slides of head and genitalia under the microscope as per method described by Lewis[17].

Bioassay on long-lasting insecticide-treated nets LN PermaNet® 2.0: Five sprayed households from each village (wards) were selected for residual efficacy of DDT by bioassay[18]. Bioassay was performed on the LN PermaNet® 2.0 provided by Vestergaard Frandsen, Switzerland (mesh size 156/inch^2 with deltamethrin @ 55 mg/m^2). Bioassay test was performed by exposing full fed female sandflies introduced into WHO plastic cones fixed on the insecticide treated surfaces for a period of 30 min after one week of indoor residual spray (IRS) (May 10, 2012). Only 20 sandflies were introduced into each cone. There were four replications of test on treated walls of the selected houses and there was a single test on untreated wall, served as a control. After exposing, the females were placed in WHO tube (10 per cup), with sucrose solution provided at screen and maintained climatic condition for 24 h at 25±2°C temperature and 80±10 per cent relative humidity. The mortality rate was recorded after 24 h. The test series having more than 20 per cent control mortality was cancelled.

Susceptibility test: Susceptibility test of *P. argentipes* against DDT was carried as per the WHO standard method[19] on DDT impregnated paper (4% DDT) provided by the Vector Control and Research Unit, School of Biological Science, University Sains Malaysia (Penang, Malaysia). The observed mortality was corrected by Abbott's formula[20].

Statistical analysis: All the statistical analysis such as mean, minimum, maximum, standard deviation were performed using the excel sheet-2007. Graph preparation and *P*-values estimation were done in SPSS software, version-16 (SPSS Inc., USA).

Results

Seasonal characteristics of sandfly: During the study period, in Maldah district two rounds of DDT spray were carried out, first in May and second round in December 2011. In 2012 only one round of DDT spray was carried out in April-May. In Burdwan district only one rounds of DDT spray each in April 2011 and December 2012 were carried out. Though the sandfly density *(P. argentipes*) was low in Burdwan, but the peak was similar *i.e.* one peak in March-April and other peak in September was observed (Fig. 1). In Maldah, in the month of November-December temperature was low, hence the collection was negligible.

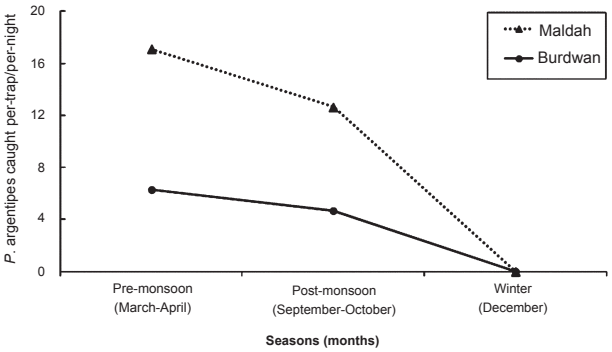

Fig. 1. Seasonal distribution of sandfly in Maldah and Burdwan districts (West Bengal, India) during the study period.

A total of 1,407 sandflies were captured during the study period. Of the collected sandflies, 69.59 per cent were *P. argentipes* (male : female ratio 1:1.15), 20.81 per cent were *Sergentomyia* (male: female ratio 1:1.11), and 9.60 per cent were *P. papatasi* (male: female ratio 1:0.74). The proportion of *P. argentipes* was significantly higher (P<0.001) compared with the proportion of *Sergentomyia* and *P. papatasi* in the study site. However, the highest density of *P. argentipes* was recorded in September and October 2011 (post-monsoon) compared with the *P. argentipes* density between March and April 2011 (pre-monsoon). The results also showed that the percentage of female *P. argentipes* was higher during the pre-monsoon and lower in the post-monsoon season. Similar results were obtained for *P. papatasi* and *Sergentomyia*. Fig. 2 shows the seasonal characteristic of abdomen stage of female *P. argentipes*. The highest percentages of fully gravid and unfed *P. argentipes* were recorded in post-monsoon (30.96%) and pre-monsoon (35.09%) seasons, respectively.

Results of bioassay on LN PermaNet® 2.0: Bioassay results on LN PermaNet® 2.0 and DDT sprayed walls in Maldah district are presented in Table I. There was 60 per cent (mean±SD, 53.33±5.77) mortality in test against LN PermaNet® 2.0 within the 3 min of exposure time. The test against DDT on wall illustrated 50 per cent (mean±SD, 60.00±10.00) mortality of sandflies with 30 min time of exposure in the study area. LN PermaNet® 2.0 showed higher efficacy by having higher mortality with low does and exposure time compared with DDT. Knockdown was nil for LN PermaNet® 2.0 and DDT after 3 and 30 min, respectively.

Results of susceptibility test: The susceptibility test was carried out in the four sets against the 4 per cent DDT impregnated paper (Table II). Each set contained 10-15 *P. argentipes*. The corrected mortality derived from Abbot's formula of *P. argentipes* varied between 40 and 61.54 per cent (mean ± SD 53.71±4.91).

Discussion

The results showed high density of *P. argentipes* in the studied area of West Bengal from March to October. Picado *et al*[21] reported high peak density in the months of May and November in Bihar. The difference may be due to climatic variations and also in IRS in the two States. In Maldah LN PermaNet® 2.0 was being used in all houses, but that also did not help in containing the sandfly density. Dinesh *et al*[22] reported that PermaNet® 2.0 and Olyset nets did not reduce the female sandfly density. However, Picado *et al*[16] reported 25 per cent reduction in *P. argentipes* population. In our study the result of bioassay with the LN PermaNet® 2.0 showed only 57.89 per cent mortality after 14-15 month of Net distribution. The results of bioassay test on the DDT sprayed surfaces demonstrated only 50 per cent mortality after two weeks of spray. In Burdwan district one round of DDT spray was done at the study sites in March 2011 and one round in June 2012. During the spot visit in March 2011 DDT mark was observed on the walls but spray was patchy. In our study, the results of the susceptibility status of *P. argentipes* against 4 per cent DDT indicated development of resistance in the vector species. The per cent corrected mortality ranged between 40-61.54 per cent. Earlier Sen[23] has reported susceptibility of sandflies to 0.25 per cent DDT. Basak and Tandon[24] have reported 100 per cent susceptibility to 4 per cent DDT impregnated papers. However, they also expressed their concern on the development of tolerance against DDT in the areas which were under regular insecticidal pressure. Hence, a large study covering the whole State with more number of *P. argentipes* is suggested to monitor the resistance in the vector species for the effective kala-azar control. For the breeding habitat the results indicated that *P. argentipes* preferred indoor breeding. In Maldah, most of the cattleshed located inside the houses provided favourable environment for breeding of *P. argentipes*. Resistance in *P. argentipes* against DDT has been reported in several parts of India[8,10]. Development of resistant in *P. argentipes* against 4 per cent DDT and low effectiveness of LN PermaNet® 2.0 in containing *P. argentipes* population are major concerns for the kala-azar control programme. To have suitable vector control strategies, regular assessment of insecticide vulnerability in kala-azar vector is desirable. The present strategy to control *L. donovani* transmission

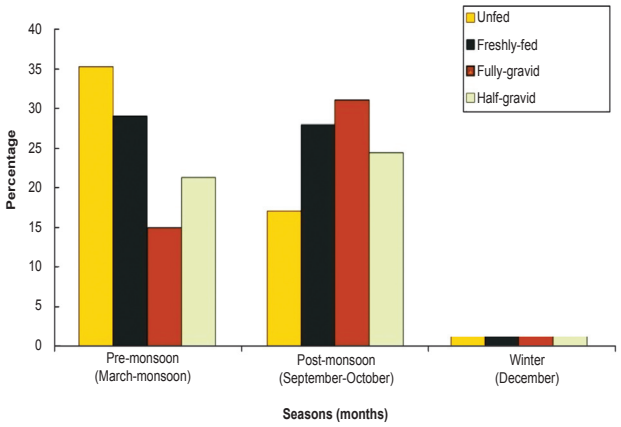

Fig. 2. Abdomen stage of female *P. argentipes* recorded during the study period (*i.e*. pre-monsoon, post-monsoon and winter seasons) in Maldah and Burdwan districts (West Bengal, India).

in the region relying on IRS with DDT should be elucidated in the light of the development of resistance in the targeted species to DDT and other insecticides. The present study indicates development of resistant in *P. argentipes* against 4 per cent DDT in the study area. Now the DDT is not as effective as observed in 1970s, when the kala-azar cases came down drastically from 1,00,000 to 11,120 in 1982[25]. Therefore, it is a matter of concern that after the development of 100 per cent resistance in *P. argentipes* against DDT the situation may worsen further.

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Table I. Bioassy test on LN PermaNet 2.0 and DDT against *P. argentipes* after 3 and 30 min of exposure, respectively, in the Maldah district, West Bengal, India

Set no.	No. of sandflies exposed	Time of exposure (min)	Knockdown after 3 min	Total mortality after 24 h	Observed mortality (%)	Corrected mortality (%)
Experiment -1						
LN PermaNet 2.0	20	3	0	12	60.00	57.89
Control	20	3	0	1	05.00	00.00
Experiment - 2						
DDT	20	30	0	10	50.00	50.00
Control	20	30	0	0	00.00	00.00

Table II. Susceptibility status of *P. argentipes* against 4% DDT in Maldah district, West Bengal, India

Set no.	No. of *P. argentipes* exposed	Knockdown (after 1 h)	Total mortality (after 24 h)	Observed mortality (%)	Corrected mortality (%)
Experimental sets (exposure time 1 h)					
Set I	15	1	10	66.66	61.54
Set II	10	0	6	60.00	60.00
Set III	10	1	4	40 .00	40.00
Set IV	15	1	8	53.33	53.33
Control sets (exposure time 1 h)					
Set I	15	0	2	13.3	0
Set II	10	0	0	0	0
Set III	10	0	0	0	0
Set IV	15	0	0	0	0

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