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RESEARCH ARTICLE

SUSCEPTIBILITY STATUS OF *PHLEBOTOMUS ARGENTIPES* TO DDT AND DELTAMENTRIN IN A FOCUS OF CUTANEOUS LEISHMANIASIS IN KANI TRIBES SETTLEMENT OF THE WESTERN GHATS IN KERALA, INDIA

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ABSTRACT

Leishmaniases are one of the neglected tropical diseases in several countries. In India, recently, cutaneous leishmaniasis cases were recorded from the tribal settlements of Western Ghats, Thiruvananthapuram district, Kerala. To provide a rational framework for selecting an appropriate insecticide for implementing intervention measure, susceptibility of sand flies of this region to DDT and Deltamenthrin was assessed. A total of 720 sandflies, comprising *Phlebotomus argentipes* (70.6%), *Sergentomyia bagdhadis* (14.4%), *Se. zeylonica* (8.2%), *P. sintoni* (3.6%) and *Se. babu* (3.2%) were exposed for susceptibility. The corrected mortality for field collected *P. argentipes* population with DDT as well as Deltamenthrin at diagnostic concentrations was 100% after 24 hr of exposure. *P. argentipes* and other sand flies were found to be susceptibility to both DDT and Deltamenthrin. Hence, DDT, which is the cheapest insecticide, is one of the options that could be effectively used as residual spraying to prevent further transmission, in the new CL focus.

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INTRODUCTION

The Sixtieth World Health Assembly, in its resolution, recognized leishmaniases as a neglected tropical disease (WHO, 2000). Leishmaniases occur in three form *viz*., visceral, cutaneous and muco-cutaneous leishmaniases. Visceral leishmaniasis (VL), also known as kala-azar is fatal, if left untreated. It is highly endemic in the Indian subcontinent and in East Africa. An estimated 200 000 to 400 000 new cases of VL occur worldwide each year. Cutaneous leishmaniasis (CL) is the most common form of leishmaniasis and about 95% of CL cases occur in the Americas, the Mediterranean basin, and the Middle East and Central Asia. An estimated 0.7 million to 1.3 million new cases occur worldwide annually (Alvar, 2012). In India, VL confined to Bihar (Prasad, 1987), West Bengal (Addy and Nandy, 1992), Jharkhand and Uttar Pradesh (Rao *et al*., 1992), is scattering to adjoining states like Gujarat and Madhya Pradesh (Dey *et al*., 2007); on the other hand, CL restricted to hot dry north western region, Thar Desert of Rajasthan (Mohan and Suri, 1975) is emerging in Himachal Pradesh (Sharma *et al*., 2003), and Kerala (Muhammed *et al*., 1990; Bora *et al*., 1996). In India, DDT is being used for the control of sandfly vectors in VL endemic areas. However, there are reports, indicating that the leishmaniasis vector, *P. argentipes* has developed resistance to DDT in a few districts of Bihar (Kisore *et al*., 2004; Singh *et al*., 2001; Mukhopadhyay *et al*, 2001). Despite, in VL endemic state in India, current vector control programme focusing on residual spraying of sand fly infested dwellings with DDT showed considerable reduction in vector density (Kumar *et al*., 2015). Recently, CL cases were recorded from the tribal settlements of Western Ghats, Thiruvananthapuram district, Kerala (Simi *et al*., 2010; VCRC, 2012). To provide a rational framework for selecting an appropriate insecticide for implementing intervention measure and to establish baseline information, susceptibility of sand flies to DDT and Deltamenthrin was assessed, following standard WHO testing procedure.

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MATERIALS AND METHODS

The study was conducted in the Kani tribe settlements ($08^0$37'49.7" North latitude-$077^0$11'29.7" East longitude; $08^0$36'51.2" North latitude $077^0$09'54.9" East longitude; with altitude, ranging from 267 to 2425 ft.). The settlements (n=28) were scattered on the southern part of the Western Ghats, montane rain forests, Thiruvananthapuram district, Kerala. Majority of the Kani tribes inhabited in mud walled huts (76.2%), while the remaining in houses made of brick and cement plastered walls, with re-in-forced concrete cement roof (23.8%).

Sandflies found resting on walls in human dwellings, tree-holes and tree-buttresses, rock holes and rodent burrows were collected using oral and mechanical aspirators, during the period from November 2013 to January 2014, between 10 00 and 12 00 hr. Specimens collected were transferred to 15x15x15cm cloth cage and fed on 10% glucose solution, soaked in cotton wool and brought to the laboratory. Healthy sandfly females with mixed abdominal stages viz., unfed, full fed and half gravid were used for assessing susceptibility to DDT and Deltamenthrin. Both DDT and Deltamenthrin impregnated and control papers were obtained from the Vector Control Research Unit, Universiti Sains, Malaysia (WHO collaborative Centre). DDT discriminating dosage, established by the WHO, for the genus Phlebotomus is 4/1 (exposition to 4% DDT impregnated paper for 1 hr) (WHO, 1981). However, no standardized discriminating concentrations or time of exposure to Deltamenthrin was given for sandflies by the WHO, as in the case for malaria vectors. The discriminating concentration ascertained by earlier works for Deltamenthrin was 0.05/1 (exposition to 0.05% Deltamenthrin impregnated paper for 1 hr) (Aboul *et al*., 1993; Tetreault *et al*., 2001; Alexander *et al*., 2009). Therefore, these diagnostic dosages of DDT and Deltamenthrin were used to assess insecticide susceptibility of sandflies in this study.

Standard WHO testing procedures were applied to assess the insecticide resistance/ susceptibility, using the test-kit tubes (WHO, 1981 and 1998). In each test, four replicates, with 20 sandflies (unidentified) were performed. Two sets of control tests were also performed, using the corresponding control papers. Sandflies were held in the test-kit tubes for 60 min. After one hour exposure, sandflies were transferred to the observation tube and kept for 24 hours, under ambient conditions of temperature (25 ± 2^0C) and relative humidity (65±10%) and maintained on 10% glucose solution, soaked in cotton wool. After 24 hour of observation, alive and dead sandflies, in each of the test-kit tube were counted and recorded. Tests were repeated 3 times with both DDT and Deltamenthrin. Sandfly specimens exposed for the bioassay were identified to species, using the standard key (Lewis, 1978). Mortality rate for sandflies was corrected according to the result of control test using the Abbott's formula (Abbott, 1925).

RESULTS AND DISCUSSION

A total of 720 sandflies comprising *Phlebotomus argentipes* (70.6%), *Sergentomyia bagdhadis* (14.4%), *Sergentomyia zeylonica* (8.2%), *Phlebotomus sintoni* (3.6%) and *Sergentomyia babu* (3.2%) were exposed for susceptibility.

The number of female sandflies exposed to test and control batches and mortality observed after 24 hr exposure were given in Table 1. The number of *P. argentipes* females exposed to DDT (4%) and Deltamenthrin (0.05%) impregnated papers were 158 and 172 respectively, as this species was abundant in the total collection. Sandfly females (n=120) were also exposed to respective control papers. The corrected mortality for field collected *P. argentipes* population with DDT as well as Deltamenthrin at diagnostic concentrations was 100% after 24 hr of exposure. The other species tested are though in small number and found to be susceptible to 4% DDT and 0.05% Deltamenthrin.

Suppression of sandfly population mainly relies on adult control. Although, DDT is being used to control sandflies in kala-azar control programme in India, particularly in Bihar, there are a few reports (Mukhopadhyay *et al*., 1992; Kumar *et al*., 2009), which indicate that *P. argentipes* has developed resistance to this insecticide. However, there was no coordinated effort to monitor resistance to insecticides. In a recent review, Ostyn *et al*. (2008) published susceptibility status of P. argentipes to DDT in the Indian subcontinent. The results of this review showed that DDT resistance has been reported in India since early 1990s. Singh *et al*. (2001) reported that *P. argentipes* was resistant to DDT in villages of Vaishali dt., in Bihar, in line with findings reported from Samstipur dt. in the same state (Kumar *et al*., 2009). Similarly, Kishore *et al*. (2004) have also reported that *P. argentipes* has developed resistance to DDT, in few districts of Bihar. Despite, in VL endemic state in India, DDT continued to be the choice of insecticide and the vector density showed considerable reduction after the spray (Dinesh *et al*., 2010). Nevertheless, for effective control, continued effort to monitor the sandfly vector resistance is essential to achieve the desired result. The CL cases are emerging in southern parts Western Ghats of Kerala (Simi *et al*., 2010) and Himachal Pradesh in northern region (Sharma *et al*., 2003). Although the morbidity associated with CL is not significant, the disfigurement and resulting social stigmatization may cause or precipitate psychological disorders, along with restricting social participation of the individuals affected by the disease. Thus, CL, like other disfiguring diseases, affects the psychological, social, and economic well-being of affected individuals (Masoom Kassi *et al*., 2008). Hence, the control of CL vector is also essential. Indigenous transmission of CL cases has been established in the Kani tribal belt, as the affected individuals have no history of movement outside the settlement area for past 2-3 years [12]. *P. argentipes* and other sandflies collected from in and around human dwellings of the Kani tribes were found to be susceptibility to both DDT and Deltamenthrin.

Table 1. Susceptibility of sandflies to DDT (4%) and Deltamenthrin (0.05%) impregnated papers

Sl. No.	Species tested	Insecticide used %	No. exposed in treated replicates	No. Dead in treated replicates after 24 hr exposure	No. exposed control	No. deadin control after 24 hr exposure	% corrected mortality
1	*P. argentipes*	DDT 4%	158	158	74	2	100
2	*P. sintoni*	DDT 4%	8	8	8	0	100
3	*S. babu*	DDT 4%	3	3	4	0	100
4	*S. bagdhadis*	DDT 4%	31	31	22	1	100
5	*S. zeylonica*	DDT 4%	40	40	12	0	100
6	*P. argentipes*	Deltamenthrin 0.05%	172	172	104	1	100
7	*P. sintoni*	Deltamenthrin 0.05%	10	10	0	0	100
8	*S. babu*	Deltamenthrin 0.05%	13	13	3	0	100
9	*S. bagdhadis*	Deltamenthrin 0.05%	45	45	6	0	100
10	*S. zeylonica*	Deltamenthrin 0.05%	Not done	Not done	7	0	Not done

Hence, DDT, which is the cheapest insecticide, is one of the options that could be effectively used as residual spraying to prevent further transmission, in the new CL

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