J Vector Borne Dis 49, December 2012, pp. 254–257

Insecticide susceptibility status of *Phlebotomus argentipes,* a vector of visceral leishmaniasis in different foci in three states of India

R.K. Singh, P.K. Mittal & R.C. Dhiman

National Institute of Malaria Research (ICMR), New Delhi, India

ABSTRACT

Background & objectives: Phlebotomus argentipes is the vector for visceral leishmaniasis in India. The development of resistance in kala-azar vector to DDT has been reported from various parts of India. The main objective of this study was to generate information on insecticides susceptibility status of *P. argentipes* to DDT, malathion and deltamethrin in different parts in three states of India.

Methods: Phlebotomus argentipes were collected from different villages, identified and used to investigate the susceptibility status against DDT, malathion and deltamethrin as per the WHO standard methods.

Results: Phlebotomus argentipes was resistant to DDT in different areas, viz. PHCs Murumgaon in Maharashtra; Ramgarh in Jharkhand; Kodah, Falka, Mahua and Lalganj in Bihar. In Phulwari Shareef PHC of Patna district in Bihar, DDT produced 89% mortality in *P. argentipes*, indicating resistant/tolerance (verification required) to DDT. The corrected percent mortality to malathion (5%) in different areas ranged between 98 and 100%; and to deltamethrin (0.05%) between 98.4 and 100%. The results showed that the tested *P. argentipes* are susceptible to malathion and deltamethrin.

Conclusion: Phlebotomus argentipes are still susceptible to malathion and deltamethrin, but resistant to DDT. The susceptibility status of *P. argentipes* should be monitored regularly in diversified situations to ascertain the judicious use of insecticides being used for indoor residual spraying in the programme for rational use of appropriate insecticide.

Key words Kala-azar; *Phlebotomus argentipes;* resistance; susceptibility

INTRODUCTION

Visceral leishmaniasis (VL) also known as kala-azar, Black fever, and Dumdum fever, first came to the attention in the year 1824 in Jessore, India (now Bangladesh), where it was initially thought to be a form of malaria. Leishmaniasis is a disease caused by protozoan parasites of the Genus-*Leishmania*. The agent of the disease was also first isolated from endemic area of West Bengal in India by Scottish Doctor William Leishman and Irish Physician Charles Donovan. The species was named after both of them *Leishmania donovani*. Today, the name kala-azar is commonly used along with the scientific name visceral leishmaniasis for the severe form of the disease caused by *L. donovani*[1].

In southeast Asia, approximately 200 million people are at the risk of VL and five lakh new cases are registered annually[2]. VL is a serious public health problem in India since 1970s, in the state of Bihar and latter spread to West Bengal and eastern Uttar Pradesh. In 1977, a sample survey estimated the number of cases to be about one lakh with 4500 deaths in Bihar[3]. The vector of leishmaniasis world over belongs to order Diptera of class Insecta (Phylum- Arthopoda). Fauna of Indian sub-zone is represented by phlebomine and sergentomyia species[4]. In India, VL caused by *L. donovani* is transmitted by *Phlebotomus argentipes*[5, 6]. About 165.4 million population and 12 states are suffering from kala-azar, of which 48 districts in four states, namely West Bengal, Bihar, Uttar-Pradesh and Jharkhand are endemic[7]. In the year 2010, 28,941 cases of kala-azar and 105 deaths were reported. The control strategy is based on the use of insecticides for reducing populations of kala-azar vector. In Bihar, two rounds of DDT spraying in a year have been conducted in kala-azar endemic districts since 1953, first round of indoor residual spraying (IRS) with DDT in February–March and second round in May–June[3,8,9]. These measures are now failing to control kala-azar in West Bengal, Bihar, Uttar Pradesh and Jharkhand states. The reason may be due resistance in *P. argentipes* to DDT used in the national vector control programme in India. A recent review on *P. argentipes* susceptibility to different insecticides in the Indian subcontinent since 1978 showed that DDT resistance has been reported in India

since early 1990's but the results were variable. Though some workers have reported development of resistance in kala-azar vector to DDT from various parts of India[3,10–20], the present study was initiated to generate more information on insecticide susceptibility status of *P. argentipes* to DDT, malathion, and deltamethrin in kala-azar endemic villages of five primary health centres (PHCs)/districts of different states, viz. Vaishali, Katihar and Patna (Bihar), Dumka (Jharkhand) and Gadchiroli (Maharashtra).

MATERIAL & METHODS

Insecticide susceptibility tests were carried out in the months of February–March 2008 in Vaishali, Patna; March–April 2008 in Katihar (Bihar); March–April 2009 in Dumka district (Jharkhand) and January–March 2010 in Gadchiroli (Maharashtra) as per the World Health Organization (WHO) procedure against various insecticides[21–22]. Sandflies were collected from unsprayed human dwellings and mixed dwellings of different villages and PHCs of different states in the early morning (0500 to 0700 hrs) with the help of suction tube and were provided 10% glucose solution soaked in cotton pads. Sandflies collected from the fields were transported in Barraud cages covered by wet cloth to the field laboratories at the PHC level for identification of *P. argentipes* which can easily be distinguished from *P. Papatasi* based on blackish body colour and silvery legs in *P. argentipes.* From a pool of collection five sandflies were killed and identified through proboscis and genitalia using identification key[4] for confirmation. After confirming *P. argentipes* the remaining population was used for susceptibility test. Insecticide impregnated and control papers for respective insecticides received from WHO Collaborating Centre at the University of Malaysia, with different diagnostic dosages were used for detection of resistance to DDT (4%), malathion (5%) and deltamethrin (0.05%).

Full-fed *P. argentipes* were exposed to WHO insecticide papers impregnated with diagnostic doses of insecticides, i.e. DDT (4%), malathion (5%) and deltamethrin (0.05%) as per the standard WHO method for one hour[22]. Temperature and humidity were controlled at 26 ± 2°C and 70–80% RH respectively. Four to five replicates containing 25 female sandflies were used simultaneously for each insecticide. Two replicates of 20–25 sandflies for control were also held parallel to each test. After exposure to the requisite period, the holding tubes were kept for recovery in dark and cool places immediately. Cotton pads soaked in 10% glucose solution were given as supplementary food during the recovery period for 24 h. The percent mortalities were calculated by scoring the dead and alive sandflies after 24 h of recovery period and mortality in sandflies was corrected by using Abbott's formula[23], in case the control mortality was within 5–20% that was expressed as corrected percent mortality.

RESULTS & DISCUSSION

Results of susceptibility tests on *P. argentipes* against DDT (4%), malathion (5%) and deltamethrin (0.05%) are given in Table 1. The corrected percent mortality of adult *P. argentipes* to DDT (4%) ranged between 31 and 89% in different areas, which indicate resistance/tolerance to DDT. Though the species was resistant to DDT in different areas in PHCs Murumgaon, Ramgarh, Kodah, Falka, Mahua and Lalganj, in Phulwari Shareef PHC of Patna district in Bihar, DDT produced 89% mortality in *P. argentipes*, indicating tolerance (verification required) to DDT. The corrected percent mortality to malathion (5%) ranged between 98 and 100%; and to deltamethrin (0.05%) between 98.4 and 100%. The results were almost same in different areas indicating that the tested *P. argentipes* are still susceptible to malathion and deltamethrin. In Murumgaon PHC of Gadchiroli district in Maharashtra, DDT resistance was very high as compared to other areas studied, showing only 31% mortality in sandflies against DDT.

DDT is still being used in the kala-azar control programme in Bihar and other endemic states. Most of earlier research workers have reported development of tolerance/resistance in sandflies against DDT from various parts of India[3,10–19]. Development of resistance to DDT in *P. argentipes* has been reported from Samastipur, Bakhtiyarpur, Dharbhanga and Vaishali districts of Bihar. However, *P. argentipes* has been reported to be susceptible to DDT in Samastipur and Patna districts[13,18]. There are only a few reports showing susceptibility status of sandflies to malathion and deltamethrin. Most of these reports have shown *P. argentipes* to be fully susceptible to malathion and deltamethrin in India[18]. However, sandflies have shown to be resistant to malathion and also deltamethrin from Puducherry, a non-endemic area for kala-azar[20]. Our study has shown resistance in *P. argentipes* to DDT only, while these are still susceptible to malathion and deltamethrin. Resistance to DDT in *P. argentipes* may be due to prolonged use of DDT for indoor residual spray since 1976 in vector control activities targeting kala-azar.

Our study reveals an increasing resistance trend in

Table 1. Susceptibility of *P. argentipes* to insecticides in different states of India

Name of state/ district/PHC	Insecticide (% conc. tested)	Sandflies exposed		Sandflies dead		% Mortality in sandflies		Corrected mortality in	Status
		Test	Control	Test	Control	Test	Control	(%)	
Maharashtra/	DDT (4)	100 (4)	25 (1)	31	0	31	0	31	R
Gadchiroli/	Malathion (5)	100 (4)	25 (1)	98	0	98	0	98	S
Murumgaon	Deltamethrin (0.05)	100 (4)	25 (1)	100	1	100	4	100	S
Jharkhand/	DDT (4)	100 (4)	45 (2)	69	0	69	0	69	R
Dumka/Ramgarh	Malathion (5)	100 (4)	45 (2)	100	0	100	0	100	S
	Deltamethrin (0.05)	100 (4)	45 (2)	100	1	100	2.2	100	S
Bihar/Katihar/	DDT (4)	125 (5)	40 (2)	83	0	66.4	0	66.4	R
Kodah, Falka	Malathion (5)	125 (5)	40 (2)	125	1	100	2.5	100	S
	Deltamethrin (0.05)	125 (5)	40 (2)	123	0	98.4	0	98.4	S
Bihar/Vaishali/	DDT (4)	100 (4)	25 (1)	61	0	61	0	61	R
Mahua, Lalganj	Malathion (5)	100 (4)	25 (1)	100	0	100	0	100	S
	Deltamethrin (0.05)	100 (4)	25 (1)	100	1	100	0	100	S
Bihar/Patna/	DDT (4)	100 (4)	25 (1)	89	0	89	4	89	VR
Phulwari Shareef	Malathion (5)	100 (4)	25 (1)	100	0	100	0	100	S
	Deltamethrin (0.05)	100 (4)	25 (1)	100	1	100	4	100	S

Figures in parentheses indicate the number of replicates; S–Susceptible (mortality ≥98%); R–Resistant (mortality ≤80%); VR–Verification required; Tolerant (81–97%).

P. argentipes to DDT but still susceptible to deltamethrin and malathion. Similar results showing 100% susceptibility of *P. argentipes* to deltamethrin and malathion have been reported by other authors from different areas[18]. The current failure to control the transmission of *L. donovani* in these districts of Bihar relying on IRS with DDT can be partially explained by the resistance to this compound and thus other insecticides should be evaluated to replace it. District-wise susceptibility status of sandflies to the insecticides being used in the programme should be monitored for judicious use of effective insecticides for kala-azar control. However, the first requirement for a successful vector control programme remains the quality of IRS implementation[24]. There is also a need to educate community for enhanced spray coverage of >85% of houses and rooms at least. There is a need to strictly monitor whether two rounds of IRS activities are done during coverage of houses.

High endemicity of kala-azar in Bihar and Jharkhand area, which is receiving two rounds of IRS with DDT, warrants that synthetic pyrethroids use should be undertaken especially in Bihar and Jharkhand states as per norms of the National Vector Borne Disease Control Programme. Given the constraints of using IRS activity for control of VL, alternative tools such as insecticidal bed nets (ITNs)/ long-lasting insecticidal nets (LLINs) may also be used. Although untreated nets may provide some degree of personnel protection against sandfly bites, but insecticide treated net[25], particularly long-lasting insecticidal nets can provide better protection against sandfly as well as control of VL[26]. There are, however, conflicting reports about the role of LLINs in control of visceral leishmaniasis vectors[25–28]. Dinesh *et al*[25] showed that LLINs although reduced the density of *P. argentipes* males but not of females, as LLINs failed to reduce the entry rate of females in selected households[27]. However, another study by Picado *et al*[26] reported that LLINs significantly reduced the density of *P. argentipes* in a village-scale trial and suggested that LLINs can be successfully deployed as part of the VL control programme[28]. Recently, a trial with deltamethrin-impregnated LLINs against *P. argentipes* has shown successful control of kala-azar in the study areas in Bihar state (unpublished personnel communication) suggesting that large-scale intervention may be undertaken with LLINs in areas with DDT resistant kala-azar vectors.

ACKNOWLEDGEMENTS

The laboratory and field assistance given by the staff of NIMR, Messrs V.P. Singh, Surender Kumar and H.K. Jha is gratefully acknowledged. Authors are also thankful to the DMOs of Vaishali, Katihar, Dumka, Gadchiroli districts and their staff for providing the information on kala-azar incidence and use of insecticide in IRS in vector control. This paper bears the NIMR publication screening committee approval No. 008/2012.

REFERENCES

1. Available from: *http://en.wikipedia.org/wiki/visceral_leishmaniasis* (Accessed on October 16, 2012).
2. Desjeux P. Leishmaniasis: Current situation and new perspectives. *Comp Immuno Microb Infect Dis* 2004; *27:* 305–18.
3. Kishore K, Kumar V, Kesari S, Dinesh DS, Kumar AJ, Das P, Bhattacharya SK. Vector control in leishmaniasis. *Indian J Med Res* 2006; *123*: 467–72.
4. Kalra NL, Bang YH. *Manual on entomology in visceral leishmaniasis*. New Delhi: World Health Organization 1988. Document SEA/VBC/35.
5. Swaminath CS, Shortt HE, Anderson LAP. Transmission of Indian kala-azar to man by the bites of *Phlebotomus argentipes*. *Indian J Med Res* 1942; *30:* 473–7.
6. Singh SP, Picado A, Boelaert M, Gidwani K, Andersen EW. The epidemiology of *Leishmania donovani* infection in high transmission foci in India. *Trop Med Int Health* 2010; *2*(15 Suppl): 12–20.
7. Available from: *http://nvbdcp.nic.in/* (Accessed on May 9, 2011).
8. *Chemical methods for the control of arthropod vectors and pests of public health importance*. Geneva: World Health Organization 1984; p. 43.
9. Kumar V, Kesari S, Dinesh DS, Tiwari AK, Kumar AJ, Kumar R, *et al.* A report on the indoor residual spraying (IRS) in the control of *Phlebotomus argentipes*, the vector of visceral leishmaniasis in Bihar (India): An initiative towards total elimination targeting 2015 (Series-1). *J Vector Borne Dis* 2009; *46:* 225–9.
10. Kaul SM, Bhatnagar VN, Mathur KK, Wattal BL. Preliminary observations on the susceptibility status *P. argentipes* and *P. papatasi* to DDT in two districts of North Bihar. *J Commun Dis* 1978; *10:* 208.
11. Joshi GC, Kaul SM, Wattal BL. Susceptibility of sand flies to organochlorine insecticides in Bihar. *J Commun Dis* 1979; *11:* 209.
12. Rahman SJ, Wattal BL, Mathur KK, Joshi GC, Kumar K. Susceptibility of laboratory strain of *P. papatasi* to organochlorine insecticides. *J Commun Dis* 1982; *14:* 122–4.
13. Mukhopadhyay AK, Saxena NB, Narasimham MV. Susceptibility of *Phlebotomus argentipes* to DDT in some kala-azar endemic area of Bihar (India). *Indian J Med Res* 1990; *91:* 458–60.
14. Joshi RD, Rai RN. Impact of DDT spraying on populations of *P. argentipes* and *P. papatasi* in Varanasi district of Uttar Pradesh. *J Commun Dis* 1994; *26:* 56–8.
15. Basak B, Tandan N. Observations on susceptibility status of *P. argentipes* to DDT in district south 24 Parganas, West Bengal. *J Commun Dis* 1995; *27:* 196–7.
16. Dhiman RC, Mittal PK. A note on susceptibility of *P. papatasi* (Scopoli) populations to insecticides. *J Commun Dis* 2000; *32*(1): 65–6.
17. Singh R, Das RK, Sharma SK. Resistance of sand flies to DDT in kala-azar endemic districts of Bihar, India. *Bull WHO* 2001; *79:* 793.
18. Dhiman RC, Raghavendra K, Kumar V, Kesari S, Kishore K. Susceptibility status of *Phlebotomus argentipes* to insecticides in Districts Vaishali and Patna (Bihar). *J Commun Dis* 2003; *35*(1): 49–51.
19. Kishore K, Kumar V, Kesari S, Bhattacharya SK, Das P. Susceptibility of *Phlebotomus argentipes* against DDT in endemic districts of North Bihar, India. *J Commun Dis* 2004; *36*: 41–4.
20. Amalraj DD, Sivagnaname N, Srinivasan R. Susceptibility of *Phlebotomus argentipes* and *P. papatasi* (Diptera: Psychodidae) to insecticides. *J Commun Dis* 1999; *31*: 177–80.
21. *Manual on practical entomology in malaria.* Pt. II: Methods and techniques. Geneva: World Health Organization 1975; p. 191.
22. *Instructions for determining the susceptibility or resistance of adult black flies, sand flies and biting midges to insecticides*. Geneva: World Health Organization 1981; WHO/VBC/81.810, p. 1–6.
23. Abbott WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol* 1925; *18:* 265–7.
24. Mondal D, Singh SP, Kumar N, Joshi A, Sundar S. Visceral leishmaniasis elimination programme in India, Bangladesh, and Nepal: Reshaping the case finding/case management strategy. *PLoS Negl Trop Dis* 2009; *3:* 355.
25. Dinesh DS, Das P, Picado A, Davies C, Speybroeck N, Ostyn B, Boelaert M, Coosemans M. Long-lasting insecticidal nets fail at household level to reduce abundance of sandfly vector *Phlebotomus argentipes* in treated houses in Bihar (India). *Trop Med Int Health* 2008; *13*(7): 953–8.
26. Picado A, Kumar V, Das M, Burniston I, Roy L, Suman R, Dinesh D, Coosemans M, Sundar S, Shreekant K, Boelaert M, Davies C, Cameron M. Effect of untreated bed nets on blood-fed *Phlebotomus argentipes* in kala-azar endemic foci in Nepal and India. *Mem Inst Oswaldo Cruz* 2009; *104*(8): 1183–6.
27. Ostyn B, Vanlerberghe V, Picado A, Dinesh DS, Sundar S, Chappuis F, Rijal S, Dujardin JC, Coosemans M, Boelaert M, Davies C. Vector control by insecticide-treated nets in the fight against visceral leishmaniasis in the Indian subcontinent, what is the evidence?. *Trop Med Int Health* 2008; *13*(8): 1073–85.
28. Picado A, Das ML, Kumar V, Kesari S, Dinesh DS, Roy L, Rijal S, Das P, Rowland M, Sundar S, Coosemans M, Boelaert M, Davies CR. Effect of village-wide use of long-lasting insecticidal nets on visceral Leishmaniasis vectors in India and Nepal: A cluster randomized trial. *PLoS Negl Trop Dis* 2010; *4*(1): e587.

Correspondence to: Dr R.K. Singh, Research Scientist, National Institute of Malaria Research, Sector 8, Dwarka, New Delhi–110 077, India. E-mail: singhriku@yahoo.co.in

Received: 21 June 2012 *Accepted in revised form:* 22 October 2012