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Insecticide susceptibility of the sand fly *Phlebotomus argentipes* in Bihar, India

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Background
Leishmania donovani is a parasite that causes the parasitic disease visceral leishmaniasis in humans (HVL), which can be fatal if left untreated. HVL control in the Indian Subcontinent (ISC) has relied on the use of insecticides for Indoor Residual Spray (IRS). Alpha-cypermethrin, a synthetic pyrethroid, has taken the place of sand flies because they are now resistant to dichloro-diphenyl-trichloroethane (DDT) [1]. Alpha-cypermethrin is used to kill sand flies, thus increasing the risk of resistance development in sand flies under the pressure of regular exposure to insecticides [2,3]. Considering the possible development of resistance in the vector *Phlebotomus argentipes*, we monitored the susceptibility status of their field populations with exploration of probable underlying resistance mechanisms in villages with and without IRS.

Materials and methods
Sand flies were collected from 10 endemic villages in Muzaffarpur district, India. These villages were categorized as continuous IRS villages and discontinuous IRS villages based on their IRS histories. CDC light traps were installed in human dwellings, cattle sheds, and mixed dwellings. Captured sand flies were brought to the laboratory at Kala azar Medical Research Center (KAMRC), Muzaffarpur. *P. argentipes* were shorted and allowed for blood feeding and rearing as described previously [4]. This study was to determine the susceptibility status of field-caught sand flies and F1 progeny against the diagnostic doses (3µg/ml for 40 min) of alpha-cypermethrin using a CDC bottle bioassay. Polymorphisms in domain II segment 6 of the voltage-gated sodium ion channel *(VGSC)* gene of pyrethroid-supreme and tolerant *P. argentipes* were detected by DNA sequencing [5].

Result
We have observed 91.19% to 99.47% knockdown mortality and 89.34% to 98.93% mortality after 24 hours from field-caught sand flies and from F1 progeny, 91.7% to 98.89% and 90.16% to 98.33%, respectively. The lowest susceptibility levels were detected in vectors collected from the Sahebganj block, whereas the highest levels were detected in the Paroo block. Two non-synonymous mutations, L1014S and L1014F, were detected, which were found to be associated with pyrethroid/organochlorine resistance. The frequency of mutation is higher in villages having continuous IRS compared to discontinuous IRS villages.

Conclusion
Populations of *P. argentipes* in Bihar are largely susceptible to synthetic pyrethroids (alpha-cypermethrin). The susceptibility status of sand flies collected from the block showed that *P. argetipes* could potentially start developing resistance. The presence of insecticide target site insensitivity in a major proportion of flies implies potential future challenges for leishmaniasis control, suggesting the need for continuous monitoring and vigilance required to sustain the elimination once achieved.

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Conflict of interest:
The authors declare no conflict of interest.

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