Establishing, expanding and certifying a closed working colony of *Phlebotomus argentipes* (Diptera: Psychodidae) for xenodiagnostic studies at the Kala Azar Medical Research Center, Muzaffarpur, Bihar, India.

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The Kala Azar Medical Research Center (KAMRC), Muzaffarpur, Bihar, India is a field station of the Institute of Medical Sciences, Banaras Hindu University. This pilot project is preliminary and essential to a larger follow-on effort aimed at defining the ability of specific human-subject groups across the infection spectrum to serve as reservoirs of *Leishmania donovani* infection to sand flies in areas of anthroponotic transmission such as Bihar state. This is possible only via xenodiagnosis of well-defined subject groups using live vector sand flies. The objective was to establish, at the KAMRC, a robust, self-sustaining, working colony of *Phlebotomus argentipes* (Annandale and Brunneti), closed to infusion with wild-caught material and certified safe for human xenodiagnostic use. Prior to this endeavor, no laboratory colony of this vector existed in India that met the stringent biosafety requirements of this human-use study. Requisite for initiating and establishing a permanent sand fly colony were: construction of a proper insectary facility, procurement of equipment and supplies, as well as training of personnel to perform the complex, labor-intensive procedures required to support the colony. All this was accomplished in seven months, March to September, 2014, under a pilot grant from the Bill and Melinda Gates Foundation. From September through mid-December, 2014, sand flies were collected from VL-endemic regions of Muzaffarpur district, Bihar (26.07uN 85.45uE) in rural villages selected previously based on reported VL cases. As a result of this first effort, a small colony was initiated and maintained for three generations but it did not achieve the critical mass necessary to be self-sustaining before the end of the collecting season. Then in March, 2015, a village was identified in which residual spraying had not been done recently and where sand fly density was consistently high enough to enable trapping large numbers of sand flies to build the colony. From March through mid-December, 2015, sand flies were collected in human dwellings and cattle sheds using 30 light traps over 254 nights (7,620 trap nights). A total of 68,601 flies was collected (37,397 males; 31,204 females). From 13,348 females set up in isoline vials for oviposition, 2,598 clutches averaging 28 eggs each were harvested, approximately 90% of which hatched). Progeny were reared according to standard methods (Lawyer et al., 1990; Modi and Rowton, 1994), providing a continuous critical mass of F1 males and females to stimulate optimal social feeding behavior. With the construction of a large feeding cage and use of a unique, custom-made rabbit restrainer, the desired level of blood-feeding on un-anesthetized rabbits was achieved for the colony to be self-sustaining and expanded to working level. Presently in its 10th generation, the colony yields 1500-2500 blood-fed females per week for egg production. Because the colonized sand flies will be used for xenodiagnosis on humans, the colony was closed to further infusion with wild-caught material in December, 2015, and steps were taken via PCR to insure the purity of the colony as *P. argentipes* and to certify it free of pathogens of potential or actual concern to humans.