Review Article

Measures to Control *Phlebotomus argentipes* and Visceral Leishmaniasis in India

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Abstract

Visceral leishmaniasis is a deadly parasitic disease that is transmitted via the bite of a female sand fly, *Phlebotomus argentipes*. The highest burden of this disease is in northern India. In 2005, India embarked on an initiative with Nepal, Bangladesh, and the World Health Organization to eliminate visceral leishmaniasis by 2015. With the goal of 1 case in 10,000 people still unmet, it is prudent to evaluate the tools that have been used thus far to reduce vector numbers and cases of the disease. Herein, we present a review of studies conducted on vector-control strategies in India to combat visceral leishmaniasis including indoor residual spraying, insecticide-treated bed nets, environmental modification, and feed-through insecticides. This review suggests that the quality of indoor residual spraying may enhance control measures while a combination of spraying, nets, and feed-through insecticides would best confront the diverse habitats of *P. argentipes*.

Keywords: Visceral leishmaniasis, *Leishmania donovani*, *Phlebotomus argentipes*, sand flies, vector control

Introduction

Leishmaniasis is a parasitic disease that can manifest in three forms: 1) mucosal, 2) cutaneous, and 3) visceral (WHO 2010, Maroli et al. 2013). Visceral leishmaniasis (VL) is the most severe form caused by the protozoan flagellate, *Leishmania donovani* (East Africa and Indian subcontinent) and *L. infantum* (also known as *L. chagasi*, found in Europe, North Africa, and Latin America). As this review focuses on elimination efforts in India, we will only refer to *L. donovani* in this article. Globally, the annual incidence rate is approximately 200,000–400,000 cases, the majority of cases are present in Bangladesh, Nepal, and India. Furthermore, two-thirds of those cases occur in India where VL (also known as kala-azar; black fever) is endemic in the states of Uttar Pradesh, Jharkhand, West Bengal, and Bihar (Alvar et al. 2006, Joshi et al. 2008, Alvar et al. 2012). Reports have noted the annual incidence in India as 146,700–282,800 cases with a mortality rate of at least 2.4%. However, other studies involving active searches at the village level have discovered mortality rates from 10–20%, partly due to a delay in diagnosis (Alvar et al. 2012). VL carries a mortality rate over 90% when left untreated (Desjeux 1996, Jeronimo et al. 2006). Transmission of *L. donovani* occurs via the bite of a female *Phlebotomus argentipes* sand fly. Of the nearly 50 species of sand flies present in India, *P. argentipes* is the only one known to transmit VL in this country (Kumar et al. 2012). Once the parasite is in the human body it rapidly invades macrophages and eventually moves in this way to the liver, spleen, and lymph nodes (Chappuis et al. 2007). Symptoms include: fever lasting weeks to months, splenomegaly, hepatomegaly, and anemia (Desjeux 1996, Guerin et al. 2002, Chappuis et al. 2007). Of patients that recov-

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er, an estimated 5–10% go on to develop a rash known as post kala-azar dermal leishmaniasis (PKDL), this can occur anywhere from several months to several years following treatment (Ramesh and Mukharjee 1995, Rahman et al. 2010). PKDL patients do not experience other symptoms outside of the rash, however, they are thought to serve as a reservoir for *L. donovani* where feeding sand flies can acquire the parasite (Addy and Nandy 1992, Rahman et al. 2010). As humans are the only known reservoir for *L. donovani* in India, PKDL patients are another concern for VL elimination efforts.

Visceral Leishmaniasis is a poverty-associated disease linked to poor housing and sanitary conditions and malnutrition, these factors have led to a number of difficulties regarding treatment and elimination (Cerf et al. 1987, Thakur 2000, Boelaert et al. 2009, Singh et al. 2010, Picado et al. 2014). Easy access to medical care in the rural VL-endemic regions of India is still limited, meaning patients are not routinely identified or wait until the disease has progressed to more severe symptoms before seeking treatment. Moreover, until recently treatment regimens required medication injections over the course of 20–28 days leading to poor compliance rates (Clem 2010, Moore and Lockwood 2010, Stockdale and Newton 2013). In addition to hurdles with medical treatment, increased sand fly density has been associated with certain types of housing, owning livestock, and nearby vegetation (Ranjan et al 2005, Singh et al. 2010, Poché et al. 2011, Poché et al. 2012, Perry et al. 2013, Malaviya et al. 2014, Picado et al. 2014). The risk of exposure to *P. argentipes* is even higher for some, as many people in these poor regions of India live in close proximity to their cattle, keeping them inside their dwellings (Singh et al. 2010, Perry et al. 2013, Malaviya et al. 2014).

As a by-product of DDT spraying for malaria elimination programs in the 1950s and 1970s, VL was nearly eliminated. However, when DDT spraying ended, sand fly numbers and cases of VL rose again, leading to several major epidemics (Kishore et al. 2006, Thakur 2007, Ostyn et al. 2008). In 2005, the governments of Bangladesh, Nepal, and India began a concerted VL elimination effort, with a target goal of 2015 for elimination (1 case in 10,000 people). Indoor residual spraying (IRS) and insecticide-treated bed nets (ITN) have been the main forms of control regimens tried in India, while environmental modification (EVM) and feed-through insecticides (FTI) have been less studied. Despite significant efforts to control sand flies and treat infected persons, the country remains a bastion for this disease. As such, we sought to review the current practices regarding vector/VL control programs in India. A literature search was performed using the term “India” in combination with the following keywords: *Phlebotomus argentipes*, sand fly, kala-azar, and/or visceral leishmaniasis. The following paper is a summation of the articles collected in hopes of highlighting what has worked, what has not, and what can be learned as we move forward towards elimination goals.

Control Strategies

Indoor Residual Spraying

IRS has been the main line of defense against vector-borne diseases in India. In the 1950s and again in the 1970s there were aggressive IRS initiatives to eliminate malaria (Kishore et al. 2006, Thakur 2007, Ostyn et al. 2008). During these years, households in malaria-endemic regions, many of which were the same as VL-endemic areas, were sprayed with DDT to cull mosquitoes. Sand fly populations declined in kind, and with them, cases of VL. However, at the end of those campaigns, with no systematic IRS programs, sand fly populations and cases of

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VL quickly rebounded (Kishore et al. 2006, Thakur 2007, Ostyn et al. 2008).

Since then, IRS using DDT has been employed to fight VL transmission in India with mixed results. Currently, the National Vector Borne Disease Control Programme of India dictates that IRS should be performed on all homes and cattle sheds in endemic regions. They recommend two annual applications, the first to occur in February/March and the second in May/June (NVBDCP 2014). Applied properly, IRS has been shown to dramatically impact *P. argentipes* density (Joshi et al. 2009). In that carefully controlled study involving all homes in six clusters in each of four villages, there was a 72.4% decline in sand fly numbers. Other studies that have assessed the efficacy of IRS in India have instead highlighted a number of issues limiting its success in controlling sand flies and VL. Huda et al. monitored local IRS programs for control of VL in Bangladesh, Nepal, and India (2011). In India, (though it was not unique for some of these issues) they found functional pumps and spare parts were lacking and 23.5% of the pumps were leaking. Spraying staff were not adequately trained and as such, proper mixing of DDT was done only 29.4% of the times observed. Additionally, proper distance and swath coverage during spraying were maintained only 49% and 58.8% of the time, respectively (Huda et al. 2011). These results are echoed in a similar report that found the same issues with equipment, training, mixing, and distance from surface, in addition to storage and quality issues for the DDT (Chowdhury et al. 2011a). This group also noted that the DDT residue levels on the walls varied at the village level from 66%–90% of the intended concentration while at the household level, the concentration varied as much as 9.1% to 330% (Chowdhury et al. 2011a). Furthermore, spraying must be applied to >80% of the homes in an area for mass effect (CDC 2012). According to Huda et al. the reported coverage rate in their study was only 64% (2011).

Another point of concern with IRS in India is resistance of *P. argentipes* to DDT. Resistance to DDT was documented for the district of Samastipur in Bihar, India as early as 1990 (Mukhopadhyay et al. 1992). In general, *P. argentipes* remains largely susceptible to DDT with the majority of flies succumbing to the insecticide (Singh et al. 2001, Dhiman et al. 2003, Joshi et al. 2009). However, select districts are showing signs of this trend shifting (Kishore et al. 2004). Huda et al. documented only a 54% mortality rate after 24 h for sand flies exposed to walls sprayed with DDT while another group found up to 70% were killed when exposed to surfaces that had been sprayed 2 weeks prior (Chowdhury et al. 2011a). It is possible that the results obtained by Huda and Chowdhury et al. may have been partly due to improper storage, mixing, spraying, and/or active ingredient concentration which were all problems documented by those studies (Chowdhury 2011a, Huda 2011a). A controlled study involving IRS in three states in India found mortality rates for *P. argentipes* ranging from 31–89%, indicating moderate to significant levels of resistance to DDT, even with proper use (Singh et al. 2012).

Despite these many problems recorded by groups studying IRS, in all of the aforementioned studies, post-treatment sand fly abundance was significantly reduced in the short term (Chowdhury et al. 2011a, Huda et al. 2011, Singh et al. 2012). In fact, Joshi et al. demonstrated a negative effect on sand fly numbers up to five months post-treatment (Joshi et al. 2009). Both Nepal and Bangladesh employ pyrethroids for their IRS regimens and have shown them to be effective (Joshi et al. 2009, Chowdhury et al. 2011a, Chowdhury et al. 2011b). Some work has been conducted using different insecticides in India. In one report they found sand flies were resistant to DDT but not deltamethrin

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(Dhiman et al. 2003). A more recent study documented that deltamethrin was effective in nearly 100% of the locations tested in India (Singh et al. 2012). The government of India is currently testing the efficacy of synthetic pyrethroids on sand fly control in Bihar (NVBDP 2014). A comprehensive table of susceptibility studies can be found in the review by Ostyn et al. (2008).

While IRS can aid in controlling endophilic sand flies, it does not address outdoor transmission of VL. In India, *P. argentipes* are peridomestic, found in cattle enclosures as well as vegetation (Poché et al. 2011, Perry et al. 2013, Poché et al. 2012). Another matter that is infrequently addressed in studies regarding IRS is the health effects to humans, animals, and the environment as a result of frequent and long-term IRS. The Stockholm Convention on persistent organic pollutants has banned DDT (van den Berg 2009). However, there is yet no consensus on whether DDT exposure leads to deleterious health effects among humans (Sharpe and Stewart 2004, Beard 2006). Regardless, there are well documented studies regarding its toxicity in birds and a variety of aquatic species (Blus 2003, Sparling 2010, Beckvar and Lotufo 2011). While further work is needed to verify them, these data indicate that alternative IRS compounds may be more efficacious in controlling *P. argentipes* abundance. Research looking at effects on human health due to long-term exposure would still need to be addressed. Ultimately, these data suggest that with proper execution, IRS could be an even more effective tool against VL in India but is not sufficient as a standalone given that *P. argentipes* is found outdoors in significant numbers.

Bed Nets

Bed nets, in particular, ITNs and long-lasting insecticide nets (LLINs), have been suggested as alternatives and/or complements to IRS for the control of sand fly populations and VL. ITN/LLINs have been effective against other vector-borne diseases, including cutaneous leishmaniasis (Lengeler 2004, Kulkarni et al. 2007, Wilson et al. 2014) but there are mixed results when it comes to sand flies in India. A trial using untreated nets found that the number of female, blood-fed *P. argentipes* declined by 85% following the introduction of the nets (Picado et al. 2009). However, the authors of that study note that they lacked concurrent controls and thus, blood-feeding rates could potentially be attributed to changes in environmental factors (temperature, humidity, precipitation) or changes in host availability (i.e. an increase in domestic animals). Even so, other groups in Bangladesh and Nepal have found similar results (Bern et al. 2000 and 2005).

Outside of that one study, all others have utilized ITNs or LLINs in India. The report by Joshi et al. investigated the usefulness of IRS, LLINs, and EVM to mitigate sand fly density in India, Nepal, and Bangladesh. When LLINs were in place, there was a village-wide reduction in *P. argentipes* numbers by 43.7% in India when measured 5 months post-intervention (Joshi et al. 2009). A subsequent village-wide study in India noted a 25% decline in sand fly density (Picado et al. 2010a). In that study, 16 clusters were enrolled, of those, 10 were used for sand fly capture studies. The reduction appeared to be at the community level and not displacement as *P. argentipes* abundance did not increase in cattle enclosures (Picado et al. 2010a). However, this observation does not exclude other environmental refuges, such as vegetation (Poché 2011, Poché 2012). As a part of the same study by Picado et al. they also investigated rates of VL in these same villages and found no protective effect of LLINs on the rate of seroconversion (Picado et al. 2010b). Nearly 20,000 people were enrolled in the seroconversion study. Over the course of 24 months, inci-

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dence of *L. donovani* infection was found to be 5.4% in the intervention group and 5.5% in the control group while clinical VL rates were 0.38% and 0.40% in the intervention and control groups, respectively. One explanation for this result was that LLINs do not prevent outdoor transmission (Picado et al. 2010b).

An earlier study of 48 homes and mixed dwellings (homes shared with livestock) in Bihar compared two types of LLINs to two types of untreated nets and looked at sand fly densities. At all time-points, up to 9 weeks post-treatment, there were no differences in *P. argentipes* abundance between the groups (Dinesh et al. 2008). It should be noted, that during the course of the aforementioned studies, unplanned IRS took place, and in both cases there was no noticeable effect on the number of sand flies captured in either study (Dinesh et al. 2008, Picado et al. 2010a). Furthermore, when the statistical models used to analyze the VL seroconversion data were adjusted for IRS, no changes were found (Picado et al. 2010b).

While the number of studies has been limited, the efficacy of bed nets in India to combat VL have not shown the same promise as they have in Bangladesh (Chowdhury et al. 2011b, Mondal et al. 2013). The potential reasons for this are many, including: study design, environmental factors (temperature, humidity, flooding etc.), and susceptibility to insecticides - *P. argentipes* in Bangladesh may be more susceptible, given their history of IRS campaigns is more recent compared to India (Bern et al. 2006, Picado et al. 2010a, Chodhury et al. 2011b). As breeding sites are yet unknown, and sand flies have been associated with vegetation in India, these remain confounding factors when accounting for differences in intervention strategies (Poché et al. 2011, Poché et al. 2012). Non-compliance and net quality are another concern. In the studies by Picado and Joshi, care was taken to inspect the nets and ensure compliance (Joshi et al. 2009, Picado et al. 2010a, 2010b). Even so, compliance with nets can be difficult as sand flies are much smaller than mosquitoes requiring finer mesh that people find stifling to sleep under in the heat of summer (Ostyn et al. 2008, Perry et al. 2013). While in theory, the impregnated insecticide should provide repellent activities, thus allowing for a larger mesh, efficacy in the field has not been shown (Picado et al. 2010a, 2010b). Moreover, 93% of 1,217 people surveyed in 2009–2011 in VL-endemic regions reported sleeping outside at some point during the hottest months of the year (Perry et al. 2013). Assuming this trend holds true throughout other VL-afflicted areas, this is a serious hurdle to be faced in regards to the efficacy of IRS, ITNs, and EVM as none mitigate transmission that occurs outside of a person’s dwelling.

Environmental Modification

Typical dwellings in the regions of India afflicted by VL are often made of mud and thatch, or brick/plaster. Some have earthen floors while others have brick or cement (Perry et al. 2013, Malaviya et al. 2014). In all cases, cracks and crevices where sand flies can rest and hide are prevalent. All home types are susceptible to habitation by *P. argentipes*, but thatched homes in particular cater to high densities (Malaviya et al. 2014). EVM is a method little studied when compared to IRS and ITNs as a means to control VL in India. EVM has generally meant alterations to the home or surrounding environment by means of covering or filling in cracks and crevices in walls and floors.

A pilot study involving 15 homes saw a reduction in sand fly numbers using a mud and lime plaster mix to seal cracks in homes and cattle enclosures (Kumar et al. 1995). The same well-controlled study comparing the efficacy of IRS and ITNs in India, Nepal, and Bangladesh found a 42% decline in sand fly abundance five months after the walls of

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homes and cattle enclosures were plastered with a mud and lime mixture. The negative effect on sand fly populations may be due to the lime pH or limiting available moisture, thus, inhibiting breeding of *P. argentipes*, though breeding sites for these flies have not been confirmed (Kumar et al. 1995, Sharma and Singh 2008, Joshi et al. 2009). Indeed, a study in Bangladesh saw no effect on sand fly populations when crevices were filled with mud (Chowdhury et al. 2011b). Importantly, another study in India found mud-plastered walls themselves to be risk factor for VL (Ranjan et al. 2005). However, while mud/lime mixes are effective, this method requires continual maintenance and is costly compared to IRS and ITNs (Das et al. 2008). But like IRS and ITN, it is only effective against the sand flies that are inside homes; populations that reside in outdoor enclosures and vegetation would remain a source of VL.

Feed-Through Insecticides

A newer, relatively untested, yet promising addition to the vector-control arsenal is the use of FTIs. Several compounds including ivermectin, fipronil, and imidacloprid have been tested in rodents to cull *P. papatasi*, a vector for cutaneous leishmaniasis (Mascari et al. 2008, Wasserberg et al. 2011, Mascari et al. 2013, Derbali et al. 2014). In these reports, the agents have been effective at controlling adult and larval sand flies when fed on blood or feces from treated animals, respectively. In regards to *P. argentipes*, ivermectin and fipronil as well as diflubenzuron and eprinomectin were tested as FTIs in rats (Ingenloff et al. 2013). In that study, fipronil at 150 ppm was shown to result in the quickest mortality and its effects had greater longevity than the other three compounds, even when they were used at greater concentrations.

Fipronil has been the only FTI further evaluated for control of *P. argentipes.*

Cattle and other livestock act as a major source of blood meals for female sand flies. Blood-meal analysis in one study found that 39.3% of the flies tested had fed on some form of livestock (Garlapati et al. 2012). So as to target *P. argentipes* that are feeding off of cattle and therefore, possibly residing outdoors, a controlled study involving cattle dosed with 0.5, 1.0, 2.0, and 4.0 mg/kg body weight of fipronil was conducted (Poché et al. 2013). That investigation demonstrated that fipronil as an FTI is effective, even at the lowest dose, at killing both adult and larval sand flies. The majority of adult sand flies had a 100% mortality rate within four days when fed on cattle up to 21 days post-treatment for all but the lowest dose (which had a ~22% mortality rate at 21 days post-treatment). Nearly all of the flies that were fed on days 1, 3, and 5 post-treatment succumbed on the same day. For larval *P. argentipes*, the mean-time to death when fed on feces from cattle 1 or 3 days post-treatment was 4.5 days, larva fed on feces with the lowest dose (0.5 mg/kg) on day 1 post-treatment had 100% mortality within 5.5 days while the highest dose (4.0 mg/kg) was 4.0 days. Additionally, for all doses tested, 100% mortality of larval sand flies was achieved by day 15.5 when fed on dung collected 21 days post-treatment (Poché et al. 2013).

Given that this method is untested under field conditions, conclusions regarding its effect on *P. argentipes* abundance or VL transmission cannot be drawn at this time. While no effects to cattle health were noted in the aforementioned study, future work will need to be done to assess the health of cattle treated long-term. Moreover, at the lowest dose, fipronil can remain in the animal for up to 35 days but it is at concentration below international allowable limits (Poché et al. 2013). Regardless, future studies should also address milk consumption by humans from these cattle. Studies regarding the efficacy of fipronil as an FTI are ongoing and if they

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prove positive, this method would be a valued addition to IRS and ITNs as it would better address exophilic *P. argentipes*, making control measures more comprehensive.

Conclusion

With humans as the only known reservoir for VL in India, rapid diagnosis and treatment would go a long way in controlling epidemics. However, if PKDL patients serve as a source of *L. donovani*, vector-control programs will also be needed to reach the goal of elimination. In this way, surveillance and reporting programs may need to be reevaluated as under-reporting of VL remains an issue within India (Mubayi et al. 2010).

Taking into account the diverse feeding and living habits of *P. argentipes*, it is likely that a combination of control measures will be necessary to eliminate VL in India. IRS, when executed properly can be highly effective (Joshi et al. 2009). Switching insecticides may be beneficial with mounting evidence of increased resistance of *P. argentipes* to DDT coupled with potential health effects on humans, animals, and the environment (Blus 2003, van den Berg 2009, Sparling 2010, Huda et al. 2011, Singh et al. 2012, Cohn et al. 2015). In the end, the efficacy of IRS is dependent on organized government programs that coordinate the spraying schedule and train the technicians, which can be either a benefit or hindrance depending on the resources available to these groups (Joshi et al. 2009, Chowdhury et al. 2011a, Huda et al. 2011). Even still, IRS works well to curb adult *P. argentipes* abundance. Efforts should also be placed on identifying breeding grounds. To this end, IRS could be focused and effective at eliminating both adult and larval flies.

Insecticide treated nets have shown limited success in India despite reports of their usefulness in other countries in combating sand fly densities (Dinesh et al. 2008, Picado et al. 2010a, 2010b). While there is some level of personal protection from sand flies afforded to people who use bed nets, more work would need to be done to confirm that the cost of these initiatives is validated. ITNs can often be more effective control measures against disease-transmitting vectors as proper use is in the hands of the affected persons instead of an outside program. That being said, non-compliance in India could be a problem as many people report sleeping outdoors during the hot summer months (Perry et al. 2013). Long-lasting insecticide nets can have repellent and insecticidal properties for years, making them a relatively cheap supplement to IRS, however, given the impoverished state of most of the afflicted regions, nets may still need to be provided by government or non-profit groups.

The most expensive of the three most studied intervention strategies is EVM (Das et al. 2008). A mud and lime mixture to seal cracks and crevices in walls and floors has shown some negative effects on sand fly abundance, yet these studies have been limited (Kumar et al. 1995, Joshi et al. 2009). Given the cost and need for continual maintenance, EVM is a strategy that may be best left for use on a case-by-case basis rather than a district-wide, vector-control measure. The state of dwellings in VL-endemic villages is a by-product of the greater issue of region-wide poverty, if that issue were better addressed, EVM would be a moot point.

The last strategy covered in this report is FTI. This relatively new addition to VL vector-control has been effective at killing both adult and larval *P. argentipes* under controlled settings (Inglenoff et al. 2013, Poché et al. 2013). At this time there is no known resistance by sand flies to fipronil and the tactic of dosing cattle begins to address outdoor transmission of VL. However, FTIs would still rely on proper usage by individuals and like ITNs, would carry similar benefits and risks of proper use. Similar to

IRS, FTIs will result in the potential for some human, animal, and environmental exposure. Although, reported residue levels of fipronil in milk is below international standards (Poché et al. 2013), long-term studies have not been conducted. Work would need to be done to monitor for any adverse effects to humans, animals, and the environment, following long-term use of FTIs to treat cattle. Should FTIs prove effective under field conditions, they would begin to fill the gap in available treatments that target sand flies outside of homes and cattle enclosures.

Similar to FTI and IRS is the use of natural botanicals as insecticides in place of synthetic chemicals. One study demonstrated that a 2% concentration of neem oil mixed with either coconut or mustard oil was effective at repelling sand flies from human subjects in India (Sharma and Dhiman 1993). While no work has been done regarding the use of potential biochemicals as insecticides in India, studies conducted in other countries on different sand fly species showed that various plant-derived compounds were effective at killing adult and larval stages of the insects (Dinesh et al. 2014). Although many of the tested biochemicals only had a ~50% mortality rate, this area of research is still relatively unexplored and may be a useful alternative to synthetic insecticides.

With millions of homes that would require intervention, vector-control programs need to balance rapid efficacy with long-term cost to ensure that if a treatment measure is terminated the country does not experience the rapid resurgence as occurred at the end of DDT IRS in the 1970s. One year of IRS costs on average, \$5.90 per household which is more than LLINs (\$4.50/house/year) but less than EVM (\$8.70/house/year), and those costs have likely only gone up since that report was conducted (Das et al. 2008). There is a real need for thorough cost-effectiveness studies using combinations of control measures and disease prevalence/sand fly population scenarios. Research documenting the direct and indirect costs, estimated expenditures for effective training and supervision of staff, as well as equipment maintenance would help to make informed decisions on the best use of resources for this endeavor.

Moreover, modelling has been done to estimate what percent of the sand fly population would need to be culled in order for VL to be eliminated. In that report, if sand fly life expectancy was reduced (eg via IRS, ITNs, or FTIs), there would need to be a 67% decline in abundance in order for VL to be eliminated (Stauch et al. 2014). Of the research conducted to date, only the FTI report and some of the IRS studies meet that threshold (Chowdhury et al. 2011a, Singh et al. 2012, Poché et al. 2013). Further focusing of efforts and resources could be done by implementing remote sensing and GIS data. Preliminary work done in Brazil and India modeling both climate and land data has been used to try and identify vector habitat as well as regions that may be the focus of VL outbreaks due to various weather and geo-environmental factors (Bhunia et al. 2013). While standardization of analysis methods and data acquisition are still needed, GIS and remote sensing could greatly help to target high-risk areas before an outbreak occurs.

Vector-control and VL-transmission studies should begin to focus on combination intervention strategies as well as enhanced public education programs. If the public are unaware of the risk, the symptoms, and treatments for VL, control programs will struggle (Singh et al. 2006, Siddigui et al. 2010, Malaviya et al 2013). From what is known at this point, IRS is efficacious in quickly curbing adult sand fly populations, ITNs may have some benefit for personal protection and might also be useful if IRS is not performed correctly 100% of the time. Lastly, FTI is deleterious to both adult and

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larval *P. argentipes* and has the potential to disrupt populations of exophilic sand flies. What has been lacking outside of the one study by Picado et al. (2010b), are studies that attempt to link a decline in sand fly abundance with a subsequent drop in cases of VL. A broader review of 84 studies in 22 countries involving all forms of human leishmaniasis found that only 35% measured *Leishmania* infection as an outcome (Stockdale and Newton 2013). This is an issue afflicting many vector/disease-control studies (Wilson et al. 2015). Future studies should concentrate their efforts on making this connection. While a decline in sand fly numbers should in theory correlate to a decline in VL infections, this link has yet to be demonstrated clearly by the research performed to date. Future work investigating the connection between reduced sand fly abundance and VL infection rates as well as shifts in parasite availability and sand fly feeding behaviors should be a priority.

In combination, these tactics may work to bring sand fly numbers down quickly in the short-term, allowing for VL-patient identification and treatment. Currently, the state of Bihar is beginning tests for use of synthetic pyrethroids in place of DDT for IRS. The national roadmap laid out by the government of India emphasizes case detection and treatment as well as surveillance for PKDL. While they state the need for integrated vector management, IRS remains the mainstay for vector control (NVBDP 2014). Ongoing work with sand fly breeding site identification, and implementation of more novel technologies like GIS and remote sensing will greatly aid in curbing this disease. The global initiative to eliminate VL in all endemic regions has been strong, however a coordinated effort between groups employing the various control tactics will be vital to see the elimination goal met. Once caseloads have been brought to 1:10,000, the use of IRS, ITNs, FTIs and all of the aforementioned technologies and trainings should be evaluated to determine which would be best for continued control measures.

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