Hurdles to achieving effective vector control for visceral leishmaniasis elimination in India

Thesis submitted in accordance with the requirements of the University of Liverpool and the Liverpool School of Tropical Medicine for the Degree of Doctor of Philosophy

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February 2022

Declaration

I hereby declare that this PhD thesis is a presentation of original work.

Material contained herein has not been previously published, accepted or presented for the award of any University degree. Every effort has been made to acknowledge the contribution of others.

Acknowledgements

First and foremost, I am extremely grateful to my supervisors, Prof. Janet Hemingway and Dr. Mark Paine for their invaluable advice, continuous support, and patience during my PhD study. I would also like to thank Dr Michael Coleman and Dr Rudra Pratap Singh, without who this work would not have been feasible, and I leave with so many wonderful memories of field work in extreme weather conditions. I would also like to thank Ms Agnes Matope and Dr Michelle Stanton for all their guidance with the statistical analysis in my thesis. From my family, I would like to express my gratitude to my parents and sister, my parents' in-law and brother-in-law for their continual support. Finally, I would like to thank my husband and two daughters, Anika and Meera for giving me endless encouragement and tremendous understanding.

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1. Introduction and Literature Review

1.1 Visceral leishmaniasis

Visceral leishmaniasis (VL), commonly referred to as kala-azar, is the most severe form of Leishmaniasis and a neglected tropical disease (NTD) of global public health importance. In 2018, the disease was reported to be endemic in 83 countries across five continents (Figure 1), with an estimated >30,000 new cases reported annually (World Health Organization, no date b). Historically, the disease has been ranked as the second largest parasitic killer globally (Chappuis *et al.*, 2007) and is caused by the *Leishmania donovani sensu stricto* complex in East Africa (Ethiopia, Kenya, South Sudan and Sudan) and South-East Asia (Bangladesh, India and Nepal). In Europe, North Africa and Latin America the causative protozoan is *Leishmania infantum* (Maurício, Stothard and Miles, 2000; Lukes *et al.*, 2007).

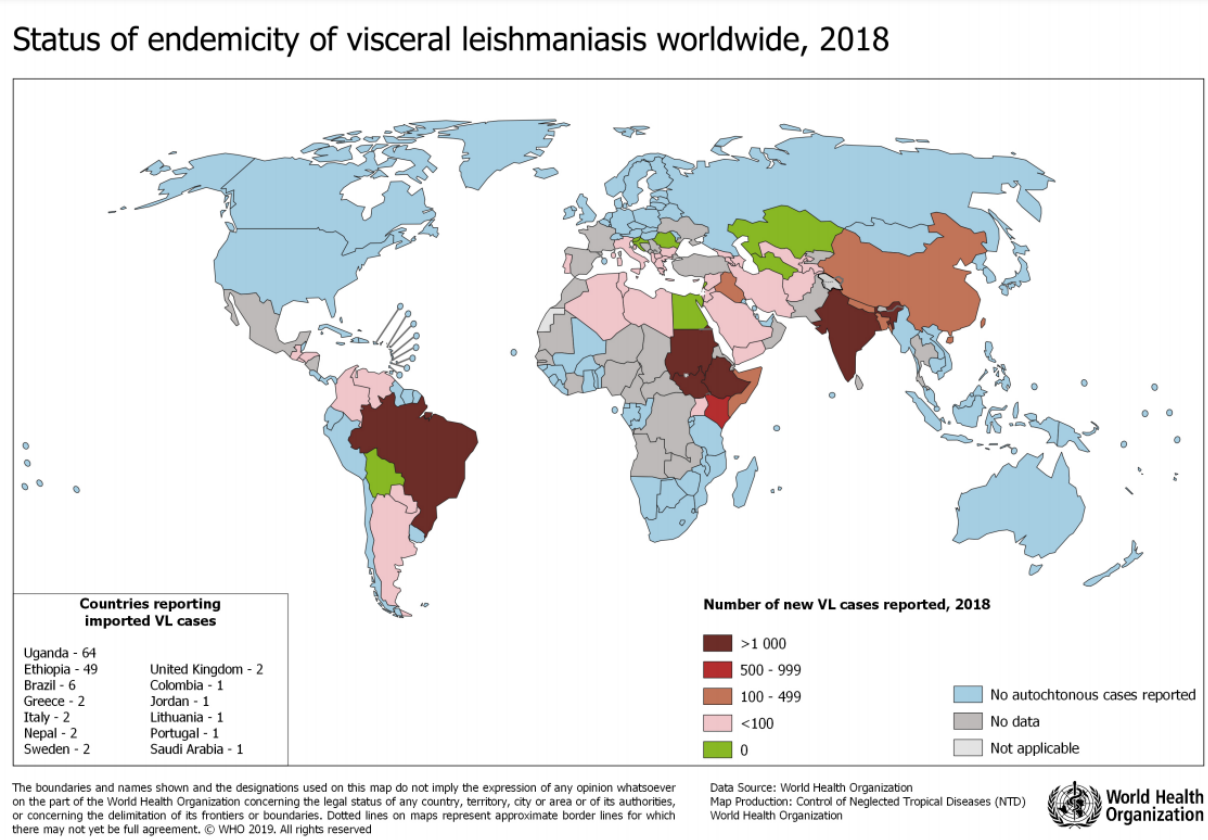

Figure 1: Status of endemicity visceral leishmaniasis worldwide, 2018 (Source: WHO)

The disease is transmitted by infective bites of female sand flies through two transmission modes; zoonotic VL is transmitted from animal to vector to human, whilst anthroponotic VL is transmitted from human to vector to human. Anthroponotic kala-azar is found in regions of *Leishmania donovani* transmission, whilst the zoonotic form is characteristically found in areas of *Leishmania infantum* (Chappuis *et al.*, 2007).

1.1.1 Life cycle

In the anthroponotic VL life cycle the infected female phlebotomine sand fly, having previously taken an infected blood meal typically 7-10 days prior (Boelaert and Sundar, 2014), injects the infective stage (promastigotes) of the flagellated protozoan into the hosts bloodstream during a blood meal. Promastigotes are phagocytosed by macrophages and other phagocytic cells in the blood. This causes the promastigotes to transform into amastigotes (tissue stage) inside the macrophages. The amastigotes multiply and infect other mononuclear phagocytic cells (Figure 2). Sand flies then ingest the amastigote infected blood cells when taking up blood meals. Inside the sand fly gut, the amastigotes transform into promastigotes and migrate to the proboscis to continue the life cycle. The human host will remain infectious to the sandfly if parasites remain in the macrophages found in the dermis, or in the circulating bloodstream (Boelaert and Sundar, 2014).

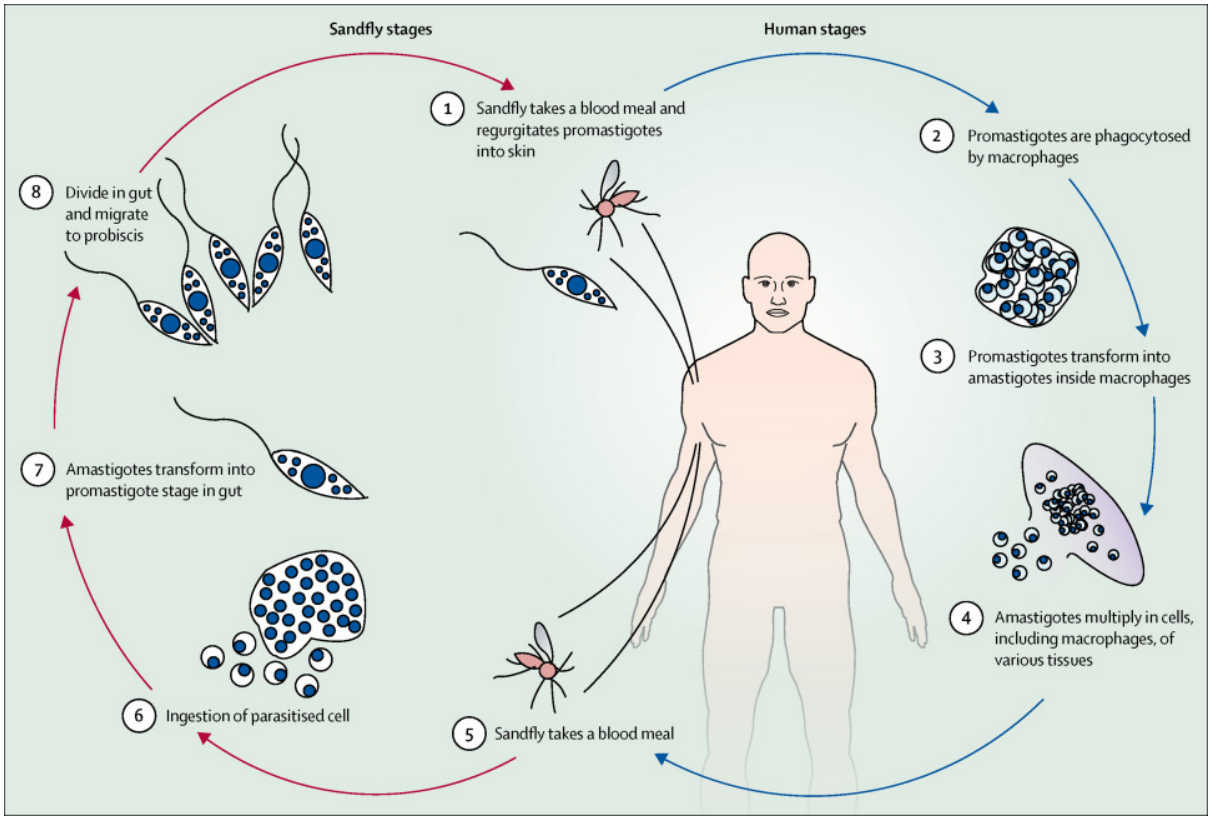

Figure 2: Anthroponotic life cycle of VL in India (Burza, Croft and Boelaert, 2018)

In zoonotic life cycles, the human stages described can also occur in animals. Zoonotic VL, caused by *Leishmania infantum* is an important disease for humans and dogs in South America and Europe (Quinnell and Courtenay, 2009). Other known reservoir hosts for zoonotic VL caused by *L. infantum* have included domestic cats and wild canids (Ready, 2014).

1.1.2 Clinical symptoms

The disease is characterised by irregular bouts of fever, severe anaemia, significant weight loss, enlarged spleen, hepatomegaly, hypergammaglobulinemia and pancytopenia. Left untreated, the fatality rate can be as high as 95% within two years (World Health Organization, no date a). Post-kala-azar dermal leishmaniasis (PKDL) is a complication of kala-azar, characterised by a macular, maculo-papular or nodular rash and is often seen after treatment in Sudan, however, it is also seen in the Indian subcontinent and other East African countries and typically appears 6 months to several years after cure of VL. Geographically there are marked differences in recovery from PKDL. Approximately 85% of cases in Africa heal spontaneously, whilst patients in India do not heal (Burza, Croft and Boelaert, 2018; World Health Organization, no date b).

1.1.3 Diagnosis

Traditionally the disease is detected through direct visualisation of the parasite using microscopy or culture of invasive samples. Across Eastern Africa and South Asia, spleen aspiration is a routine procedure. This method is highly specific and provides relatively high sensitivity (best performance: 93-99%) (Srivastava *et al.*, 2011; Burza, Croft and Boelaert, 2018). Other types of aspiration can include bone marrow and lymph nodes, however these have lower-levels of sensitivity (van Griensven and Diro, 2019). The use of microscopy methods requires well-trained staff. In countries where VL is rarely seen, correct diagnosis by microscopy can be problematic (van Griensven and Diro, 2019). Antibody-detecting rapid diagnostic tests (RDTs) using the K39 protein are cheap, easy to use and perform well in the South Asian region (sensitivity of 97%). They currently form the cornerstone of diagnosis in the regional VL elimination programme (Matlashewski *et al.*, 2013; Boelaert *et al.*, 2014). Other accepted methods of diagnosis include enzyme-linked immunosorbent assays, immunofluorescence antibody tests, direct agglutination tests and molecular tests such as polymerase chain reaction, however, these are not widely implemented at scale within programmes. Diagnosis of VL in immunocompromised patients is challenging, due to parasites being present in atypical locations (e.g. intestinal or oral ulcers) and in these patients spleen or bone marrow aspiration can be negative. In addition, serological tests have been reported to be less sensitive in immunocompromised patients.

1.1.4 Treatment

Treatment of all symptomatic VL with anti-leishmanial drugs is essential, as left untreated it will nearly always be fatal. Treatment regimens for *L. donovani* and *L. infantum* VL includes the administration of liposomal amphotericin B, miltefosine, paromomycin, amphotericin B deoxycholate and pentavalent antimonials (Directorate National Vector Borne Disease Control Programme, 2017; van Griensven and Diro, 2019).

1.1.5 Vector

The vectors of leishmaniasis are species and subspecies of *Phlebotomus* in the Old World, and *Lutzomyia* in the New World (Assimina, Charilaos and Fotoula, 2008; Dawit, Girma and Simenew, 2013). Both male and female sand flies feed on plant sugars, however the female requires a blood meal to lay eggs. The larval development stages can range from 30-60days and take place in moist microhabitats rich in organic matter (Boelaert and Sundar, 2014). However, the exact location of breeding sites for the majority of sand fly species are unknown and this is problematic when trying to achieve targeted adult stage vector control (Boelaert and Sundar, 2014).

Classed as weak flyers, sand flies usually fly close to the ground in short hops. Their flight range is typically around 300m, however in desert environments they have been known to fly up to 2300m (Goddard and Zhou, 2007; Calborn, 2010). Adult sand flies tend to be restricted to the general vicinity to the larval development site, which is usually organically rich and moist.

Sand flies are capable of adapting to environmental changes and spreading to new geographical areas, causing the pattern of transmission of VL to change over time (Calborn, 2010). However, sand flies are also very susceptible to dehydration, therefore most are nocturnal and seek shelter in cool, dark sheltered places such as animal burrows, tree buttresses, holes, caves, rock crevices, termite hills and inside human habitations (Calborn, 2010; Boelaert and Sundar, 2014) during the day. In the New World, sand flies are often found near tree buttresses and caves, whereas in the Old World they are typically found in contaminated soils of domesticated animal shelters, termite mounds, rodent burrows and earthen floors of human habitations (Feliciangeli, 2004).

The sole species of sand fly transmitting VL in India, Nepal and Bangladesh is *Phlebotomus argentipes*, whilst in other regions the vector species include flies from the Lutzomyia and Phlebotomus genera (Boelaert and Sundar, 2014; Dodd and Stramer, 2017).

1.1.6 Vector control

In order to significantly reduce disease risk, vector control methods are often introduced within VL elimination efforts. Adopting many practices commonly used to achieve effective malaria vector control, efforts primarily focus on strategies targeting adult stages. The most useful and utilised method is indoor residual spraying (IRS).

1.1.6.1 Indoor Residual Spraying

Dichlorodiphenyltrichloroethane (DDT) was the first insecticide used in efforts to control sand flies in Peru in the Rimac Valley (1944) by Hertig & Fairchild (Fairchild and Hertig, 1948; Alexander and Maroli, 2003). This was followed by field trials in Palestine, Italy and Greece (Hertig and Fisher, 1945; Jacusiel,

1947; Hertig, 1949). The early field trials established the four core principles of sand fly control (Fairchild and Hertig, 1948; Alexander and Maroli, 2003):

1. Residual spraying of houses and animal shelters are effective at preventing sand flies from taking a blood-meal
2. This method of vector control eliminated resting and breeding sites by restricting sand fly access to suitable areas
3. Spraying provides a localised effect, therefore control measures need only to be undertaken within a limited area (few hundred metres)
4. The relatively long lifecycle of sand flies meant a delayed recovery of populations within a treated area.

Broad scale control of sand flies, was achieved using large quantities of DDT in India, Brazil and the People's Republic of China (Alexander and Maroli, 2003). One reported success in sand fly control, although circumstantial, was provided by the Indian National Malaria Eradication Programme between 1958 and 1970: during this time no cases of VL were reported in the State of Bihar, as house-spraying efforts targeting the malaria vector *Anopheles culicifacies* also effectively suppressed *P.argentipes* populations. However, this effect was short-lived as within months of the programme being halted PKDL cases appeared, and between 1977 and 1990, 301,076 cases were reported with a fatality rate of 2% (Thakur and Kumar, 1992). This collateral benefit to malaria control was also noted in Bangladesh (Elias, Rahman and Khan, 1989), Syria (Tayeh *et al.*, 1997) and Peru (Davies *et al.*, 1994).

The historical success of IRS in India to control *P. argentipes* to disrupt VL transmission has been reported using DDT in Uttar Pradesh (Joshi and Rai, 1994) and West Bengal (Mukhopadhyay *et al.*, 1996), whilst malathion was reported to be effective in Gujarat (Pandya, 1983). Studies in Algeria (Alexander and Maroli, 2003) reported a rapid decline in annual incidence of leishmaniasis cases after one year of DDT IRS.

The Stockholm Convention (UNEP, 2009) has identified 12 persistent organic pollutants (POPs) including DDT which cause adverse effects on humans and the ecosystem, and bio-accumulate in living organisms. The POPs are placed into three categories: pesticides, industrial chemicals and by-products, of which DDT sits within the pesticide category. More specifically, DDT is listed in the Stockholm Convention to restrict its use within public health only where locally safe, effective and affordable alternatives are not available. India ratified the Stockholm Convention on 13th January 2006, which demonstrated the country's commitment to meet international obligations related to protection of human health and the environment (Government of India, 2020a). Despite this commitment, India remains one of the main global manufacturers of DDT, continues to use DDT within

its own malaria control programme, and still supplies the insecticide for public health control to countries such as South Africa, Zimbabwe and Zambia (Government of India, 2020b).

Today a range of different insecticides are used within IRS programmes globally for leishmaniasis control, including alpha-cypermethrin (Faraj *et al.*, 2013), deltamethrin (Kayedi *et al.*, 2017), lambda-cyhalothrin (Dora Feliciangeli *et al.*, 2003), bendiocarb and pirimiphos-methyl (Coulibaly *et al.*, 2018).

1.1.6.2 Insecticide treated nets and Long-lasting insecticide treated nets

Historically, insecticide treated nets have been evaluated against phlebotomine sand flies in leishmaniasis endemic countries including Italy (Maroli and Lane, 1989), Burkina Faso (Majori et al., 1989), Syria (Desjeux, P, 2000), Sudan (Elnaiem, Elnahas and Aboud, 1999; Elnaiem et al., 1999), Kenya (Mutinga et al., 1992, 1993), Colombia (Alexander et al., 1995) and Venezuela (Kroeger, Avila and Morison, 2002). The advantages of insecticide treated bed nets are efficacy, cost and sustainability (Alexander and Maroli, 2003), but there are concerns over net mesh size – as the nets are primarily designed to control mosquitoes, and a change in sand fly feeding behaviour to more exophilic and exophagic patterns (Dinesh et al., 2008; Poché et al., 2012). The change in behavioural resistance has potential implications on sand fly control and the effectiveness of strategies implemented, however there is limited reliable information on important aspects sand fly ecology in India, including natural oviposition habitats and sugar sources (Warburg and Faiman, 2011; POCHÉ *et al.*, 2017).

The KALANET community trial in 2006, conducted in India and Nepal (Picado et al., 2010) demonstrated that there was no evidence that large-scale distribution of long-lasting insecticidal nets provided additional protection against visceral leishmaniasis when compared to the pre-existing practices within the Indian subcontinent. Conversely, a trial in Bangladesh conducted between 2006-2019, found community-wide insecticide impregnation of existing bed-nets reduced VL incidence by 66.5% (Mondal et al., 2013). The latter study findings could be influenced by technical factors including, the type of nets and insecticides used, lack of replicates and issues with randomisation (Picado et al., 2015). In addition, the different results from the two studies could be due to biological factors such as the susceptibility status of sand flies and sand fly behaviour (Picado et al., 2015). Finally, an observational study to determine the effect of untreated bed nets on blood-fed *P. argentipes* vectors in Nepal and India showed a reduced blood feeding rate of 85% (95% CI 76.5-91.1%), which provided circumstantial evidence that untreated nets provided some personal protection against sand fly bites (Picado *et al.*, 2009).

1.1.7 Global targets

VL was included within the initial 2012 WHO road map for accelerating the reduction of global impact of 17 NTDs by 2020 (World Health Organization, 2012a), and was subsequently included in the WHO

2021-2030 blueprint to accelerate progress towards prevention, control, elimination and eradication of 20 NTDs and reaching Sustainability Development Goals (World Health Organization, 2020a). As per the 2012 road map, the target for VL elimination was less than 1 case per 10,000 population at district and sub-district levels (World Health Organization, 2012a). However, an updated global target to eliminate VL as a public health problem by 2030 has been defined as <1% case fatality rate due to primary disease with 85% of countries achieving this target by 2030 (World Health Organization, 2020a).

The successes to date noted by WHO include the "reduction in number of cases reported annually in South-East Asia from more than 50,000 cases to fewer than 5,000 in 2018", whereby the majority of cases (93%) were reported in India and 7% from Bangladesh and Nepal (World Health Organization, 2020a). However, it has also been reported that up to 75% of households affected by VL in Bangladesh, India, Nepal and Sudan are impacted financially in obtaining access to diagnosis and treatment, despite tests and medicines being nominally free of charge (Anoopa Sharma *et al.*, 2006; Sundar *et al.*, 2010; Ozaki *et al.*, 2011; Meheus *et al.*, 2013; Uranw *et al.*, 2013).

1.2 Visceral leishmaniasis in India

1.2.1 Historical Review - Bihar (1937-2014)

A historical review of VL in Bihar, can be found in Chapter 2. This chapter has also been published on the Gates Open Research platform, reference below:

2018, Deb RM, Stanton MC, Foster GM *et al.* **Visceral leishmaniasis cyclical trends in Bihar, India – implications for the elimination programme.** [version 1; referees: 1 approved, 2 approved with reservations] Gates Open Research 2018, 2:10 (doi: 10.12688/gatesopenres.12793.1)

1.2.2 2014-2021

2014 studies by Coleman et al. (Coleman *et al.*, 2015) highlighted the high levels of DDT resistance present in the wild population of *P. argentipes* sand flies in India and low residual concentrations (0.37 g ai/m2) of DDT detected post-IRS, which led to a national policy change for VL elimination. Alpha-cypermethrin was approved for IRS use in the VL programme and compression pumps replaced the previously used stirrup pumps (Kumar *et al.*, 2020). Despite the well documented evidence to demonstrate potential for cross resistance between DDT and pyrethroid insecticides (Amin and Hemingway, 1989; Reimer *et al.*, 2008; Hancock *et al.*, 2018), the decision of what insecticide to switch to was government led and driven by the national government approved list of insecticides for use in public health vector interventions in India. Furthermore, the importance of regular IRS quality assurance and entomological monitoring was stressed, as the study also highlighted the presence of DDT resistance as early as 1993 (Coleman *et al.*, 2015). Despite concerted efforts to achieve

elimination by 2020 (Mondal *et al.*, 2009a; World Health Organization, 2016c), VL elimination was not achieved, and the target date was further delayed. The recent delay in achieving elimination may be attributed to the global Coronavirus pandemic and a prolonged national lockdown (BBC, 2020; The Lancet, 2020) as IRS efforts were halted during this period.

1.3 Indoor residual spraying

Indoor residual spraying (IRS) is the primary vector control intervention used to control *P. argentipes* populations across all VL endemic areas in India (Directorate National Vector Borne Disease Control Programme, no date a). As *per* WHO guidelines (World Health Organization, 2015), the strategy is appropriate to use in environments where:

1. The vector population largely feeds and rests indoors (houses and other structures)
2. The human population predominantly sleep indoors at night
3. The majority of the structures are suitable for spraying
4. The vector is susceptible to the insecticides available for IRS
5. The vector-borne disease transmission pattern is such that the population would be protected by two rounds of IRS per annum
6. The strategy would be cost effective, when considering distribution of structures within a given area and associated transport costs for the intervention.

The IRS strategy aims to reduce and ultimately stop the transmission of a vector-borne disease by reducing the survivorship of the vector, its density and the human-vector contact: it is a strategy that is safe for human health and the environment (World Health Organization, 2015).

1.3.1 IRS for VL elimination in India

Spray efforts were first started as part of the National Malaria Control Programme in the 1940s and later as the National Malaria Eradication Programme (Deb *et al.*, 2018). As noted in section 1.2.1, national vector control efforts were not consistent and often occurred as a reactive response to high case burden reports. Concerted vector-control efforts for VL restarted in 2005, however the elimination target was moved several times, finally to be in alignment with the global elimination target of 2030 (NTD Modelling Consortium Visceral Leishmaniasis Group, 2019). The insecticide of choice until 2014 was DDT, however in light of reports demonstrating high levels of resistance, this was changed to alpha-cypermethrin in 2015 (Coleman *et al.*, 2015; National Vector Borne Disease Control Programme, 2015). Until 2016, the IRS programme conducted spray activities using stirrup pumps, however after support from the Liverpool School of Tropical Medicine and Bill and Melinda Gates Foundation to procure hand-compression pumps, the stirrup pumps were replaced.

Vector-control interventions are coordinated and managed by the Ministry of Health and Family Welfare through the National Vector Borne Disease Control Programme (NVBDCP). Implementation of the IRS strategies is run at state level by the State Programme Officer based within the Ministry of Health and Family Welfare State level office, with IRS plans produced at the district level. Annually two rounds of IRS are conducted (February-March, May-June), targeting all walls within house and animal structures; spraying up to a height of six feet, to ensure optimal protection for the at-risk population during peak times of VL transmission (National Vector Borne Disease Control Programme, 2015). The average house size in areas targeted for IRS is estimated to be 75m^2.

1.3.1.1 IRS Pumps in India

The Indian IRS programme used stirrup pumps for all IRS activities until 2016. The pump apparatus is composed of a 15-litre bucket, pump and a spray lance with nozzle, as shown in Figure 3 (World Health Organization, 2010, 2015; Yewhalaw *et al.*, 2017). The pump is reliant on two operators to conduct IRS, one holding the lance to control the direction of insecticide solution flow, and the other to operate the pump and provide even pressure to suck the insecticide solution from the bucket into the lance (National Vector Borne Disease Control Programme, 2015).

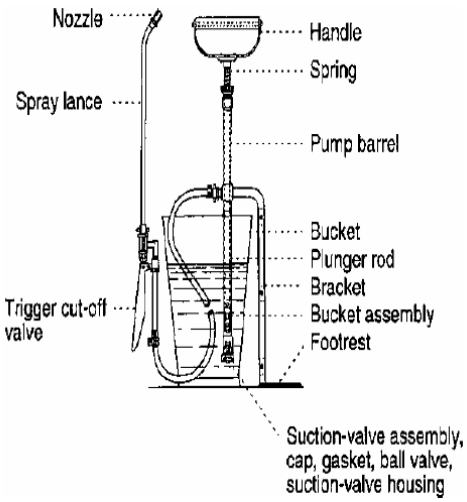

Figure 3: Stirrup Pump (Source: WHO)

The Hudson X-Pert Stainless-Steel Sprayers (hand-compression pumps) provided to the Indian IRS programme have a capacity of 11.4 litres (allowing for 7.5 litres water and 3.9 litres air space to create pressure) and include a control-flow value (CFV). When the pump is pressurised at 58psi as per WHO guidelines for IRS, with the valve 30ml of liquid is used to cover 1m^2, conversely without the CFV, 40ml is required to cover 1m^2. As shown in Figure 4, the components include a closed tank that can be pressurised, pressure gauge, hose, CFV and an 8002E nozzle. To operate the pump one person is required, who can pressurize the pump, then carry it on their shoulder to control the lance during IRS: such pumps are widely used within public health IRS programmes globally.

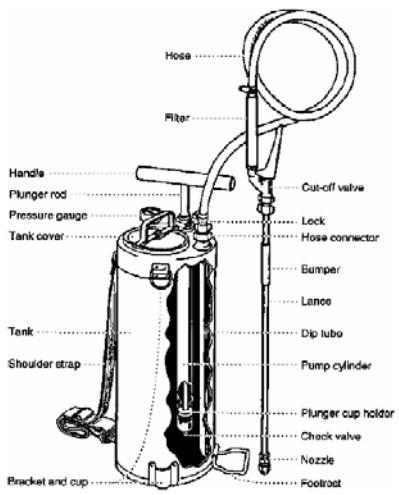

Figure 4: Hand compression pump (Source: WHO)

1.3.1.2 Spray Technique

According to WHO guidelines, when spraying vertical swathes 75cm wide should be applied to the surface, with a 5cm overlap (Figure 5) (World Health Organization, 2007). To maintain an accurate swathe width, the spray tip should be kept at 45cm from the wall at all times and the spray speed should cover one metre every 2.2 seconds (World Health Organization, 2015).

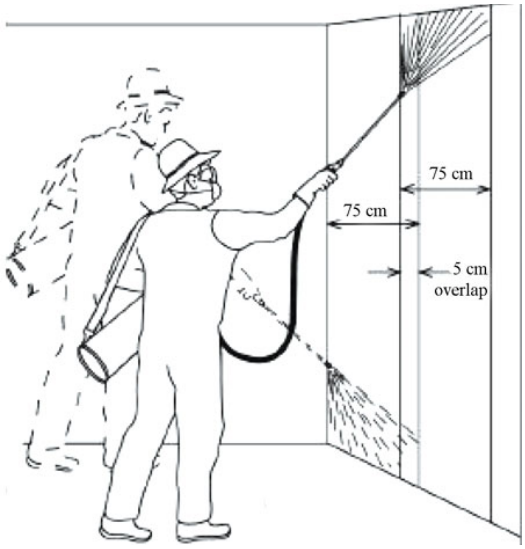

Figure 5: Schema to show how to perform IRS (Source: WHO)

1.3.1.3 Insecticides

Since the 1940s, DDT 50% wettable powder (WP) has been used for IRS against VL in India with a target delivery dose of 1g per square metre. Per house the average requirement of DDT is estimated to be 150g. In order to protect one million at-risk people, NVBDCP calculations outline that 37.5 metric tonnes of DDT are required per spray round (National Vector Borne Disease Control Programme,

2015). When using the stirrup pump, 1kg of DDT 50% WP is added to 10 litres of water (National Vector Borne Disease Control Programme, 2015). For the hand-compression pump with CFV, a total of 500g of DDT 50% WP plus 7.5 litres of water is required to achieve the target dose (National Vector Borne Disease Control Programme, 2015).

In response to reports demonstrating high levels of resistance, IRS with alpha-cypermethrin 5% was introduced in 2015, spraying a target dose of 0.025g per square metre. To achieve the target dose when using stirrup pump, 250g of alpha-cypermethrin 5% is added to 10 litres of water (National Vector Borne Disease Control Programme, 2015). However, when using the hand-compression pump to deliver the insecticide, 125g of insecticide is added to 7.5 litres of water.

1.4 Indoor residual spraying performance and impact monitoring

The key indicators outlined by WHO for continually assessing the performance and impact of IRS include (World Health Organization, 2010, 2015; Yewhalaw *et al.*, 2017):

- Performance indicators:
    - Access and coverage achieved during IRS
    - Residual Activity
        - World Health Organization Cone Bioassays
    - Verification that the target dose of insecticide has been sprayed

- Impact indicators:
    - Entomological surveys to determine:
        - Vector species
        - Seasonal density and distribution of the vector
        - Resting and feeding behaviour of the vector
        - Insecticide susceptibility status
    - Xeno-monitoring:
        - Determine transmission in vector (parasite presence)

1.4.1 Indicators to measure IRS performance

1.4.1.1 Access and coverage

If the coverage of IRS increases, the mass effect on the adult vector population and therefore protection provided to the people within the community should increase. The strategy is most effective where local vectors demonstrate endophagic and endophilic behaviour, however, it would also be expected to have some impact where vectors often feed and rest outdoors (Russell *et al.*, 2016).

For IRS efforts to be considered effective within a malaria elimination environment, high coverage (above 85%) of IRS should be achieved on all structures identified as potential resting places and to obtain the "mass effect" on the vector population (World Health Organization, 2015). As per WHO guidelines, a minimum of 80% coverage of targeted structures, houses and population at risk is targeted (World Health Organization, 2015). In order to track access and coverage, daily, weekly and monthly reporting should be conducted on spray operations for effective structure, house and population coverage indicator monitoring. In addition to the spray associated data collection, random sample surveys should be conducted to cross-check data validity of the reported coverage. Targets should be established for the number of houses or rooms to which coverage can be tracked against and where operational shortcomings are identified, action should be taken to overcome constraints and achieve high coverage. In addition to noting the structures that were effectively sprayed, capturing structures that have been missed or closed, or have been re-plastered should also be noted to fully understand coverage data and allow for remedial action e.g. mop up spray efforts.

To calculate coverage, the total number of structures sprayed during the time period of activities (day, week, month or round) is divided by the target total number of structures scheduled to be sprayed during the time period (World Health Organization, 2015).

1.4.1.1.1 Coverage data collection for VL in India

Within the Monitoring and Evaluation tool kit for IRS, developed by WHO TDR for VL elimination in Bangladesh, India and Nepal, monitoring coverage at the household level is a requirement. In India, programmatic level guidelines developed for malaria have been transferred for use in the VL IRS efforts. Coverage data fields have been modified to a more country appropriate level of granularity, whereby structures are disaggregated to demonstrate coverage achieved in rooms, verandas and cattle sheds. The target for number of sprayed structures is set at 60-80 per team per day (Chowdhury *et al.*, 2011), and data is captured on whether a house is sprayed, refused or locked (Directorate National Vector Borne Disease Control Programme, no date b). In addition, guidelines advise that five minutes would be required to spray a typical house with a surface area of 150m^2.

1.4.1.2 Residual Activity

Understanding the persistence of an insecticide on a sprayed surface is essential to determine the length of time that the product will be effective at providing protection. Where insecticides used in IRS aim to provide long-acting effect on a surface, and a highly toxic effect on vector insects – the residuality of the active ingredient should be long enough to cover the transmission season (World Health Organization, 2015). It has been reported that insecticides last longer on wood and thatch surfaces in comparison to mud, cement, concrete and brick where absorption and chemical decomposition have been observed (World Health Organization, 2015). It is estimated that the

residual efficacy of insecticide on absorbent surfaces can be 10-20% less than on non-absorbent surfaces, which further emphasises the importance of the correct dose of insecticide to be sprayed on surfaces (World Health Organization, 2015). Multi-country studies considering the residual activity life of insecticides on different surface types, with WHO standard cone bioassays, have demonstrated significant differences in effective residual due to surface type notably when using bendiocarb, pirimiphos-methyl CS (Rowland *et al.*, 2013; Oxborough *et al.*, 2014; Dengela *et al.*, 2018), however this trend was not ubiquitous across all surface types and countries (Maharaj *et al.*, 2004; Dengela *et al.*, 2018). The understanding of why residual activity life is affected by surface type is limited, and further investigation is required to support and inform IRS programming.

To determine the efficacy and residual effect of insecticide sprayed on target surfaces, and relate this to the quality of spray application, WHO cone bioassays should be conducted (World Health Organization, 2006a, 2015). This should ideally be performed on susceptible strains of the vector from insectary colonies, however where adequate resources are absent, field-collected susceptible vectors could be used. In the 2015 WHO guidelines, references to alternative colorimetric assays, still under development, are made to quantify the amount of insecticide on the wall surface (World Health Organization, 2006a). This thesis will look to provide evidence on the suitability of colorimetric assays under development for use in areas where DDT is used.

1.4.1.2.1 Residual activity data collection for VL in India

Guidelines for monitoring the bio-efficacy of the insecticide is included within the WHO TDR guidelines, whereby measurements should be taken at the two-to-four-week time point and then five months after IRS has been conducted in places where vector collections using CDC light traps are performed (World Health Organization, 2010). Whilst the guidelines have been amended to reflect the VL vector of sand flies, the procedure for conducting the test is the same as used within malaria control programmes for residual activity monitoring.

1.4.1.3 Verification of insecticide dose sprayed

The quality assurance of IRS should be surveyed routinely to ensure that the recommended dose of insecticide has been applied to sprayed surfaces. The method to assess accuracy of IRS requires at least four 5cm x 5cm Whatman Grade 1 filter papers to be attached onto different walls at various heights before IRS is conducted. After spraying the filter papers are left to dry, and then removed for chemical analysis. Once analysed, the insecticide concentration detected on the filter paper is reported in mg/ m^2 (World Health Organization, 2006a).

1.4.1.3.1 Verification of insecticide dose sprayed in India

The WHO TDR document designed specifically for supporting VL elimination outlines the same methodology to that for resources suitable for malaria endemic regions. In addition to the filter

papers, mimic and coloured papers of the same size are affixed onto the wall to minimise bias from the spray operator, who may be aware that their performance is being assessed (World Health Organization, 2010).

1.4.2 Indicators to measure IRS impact

Where the disease control or elimination strategy includes vector control, monitoring the effect on the target vector population is crucial. This is particularly relevant where interventions are insecticide-based and target the adult stages, namely IRS or long-lasting insecticidal nets.

1.4.2.1 Vector species

Studies to identify primary and secondary (where applicable) vectors should be conducted to determine the species responsible for transmission (World Health Organization, 2015). Whilst globally there are many VL vectors, in India, the vector is only the *P. argentipes* sand fly (World Health Organization, 2010).

1.4.2.2 Seasonal density and distribution of the vector

Understanding the abundance of vectors where interventions have been placed is an essential part of IRS monitoring. Indoor residual spraying takes advantage of vectors searching for blood meals indoors, within human habitations or animal shelters. Prior to, or after the blood meal, the vector rests on the walls, ceilings, and other interior surfaces, when these are sprayed the vector will absorb a lethal dose of insecticide, which will reduce its lifespan (World Health Organization, 2015). The progressive reduction in vector density and longevity should lead to an overall reduction in vectoral capacity and reduction in disease transmission, and monitoring this impact is essential.

Whilst the method or frequency to monitor this is not defined in WHO guidelines, the guidelines advise that the most appropriate method and frequency, relevant to the vector behaviour, should be used (World Health Organization, 2015). Vector collection methods will collect a number of different insects, therefore identification of the vector species', then their sex, physiological status and parity should be conducted where possible (World Health Organization, 2015).

Guidelines published in 2018 by the VectorNet project outlined two key collection methods for monitoring abundance of flying sand flies: light traps and sticky traps (Medlock *et al.*, 2018).

Relative abundance can be used when collection methods are standardised and therefore spatial and temporal comparisons can be made. When using a collection method, it is noted that vectors collected would be a sample of the population (European Centre for Disease Prevention and Control. and European Food Safety Authority., 2018).

The formula for calculating abundance is:

$$Total\ number\ of\ vectors\ collected / Total\ number\ of\ collections$$

Relative abundance is expressed as the numbers per standardised sample (Medlock *et al.*, 2018). Where collections are conducted for multiple time points, the abundance would be expressed as "number of vectors per collection method per time point".

To determine distribution of the vector, collections need to be conducted to assess presence across a region, this could be across a country or region dependent on the public health requirements.

1.4.2.2.1 Vector density for VL in India

The WHO TDR guidelines to support VL elimination in Bangladesh, Nepal and India provide clear guidelines including timepoints and methods to assess vector density; whereby the focus is to determine the impact of IRS, rather than determining the seasonal density or distribution as this is considered to be widely understood and appropriately characterised.

CDC light trap collections, should be made 2-4 weeks prior to IRS, followed by 2-4 weeks and 3-4 months after IRS. Each collection should be for one night per time point (World Health Organization, 2010). The guidelines advise the inclusion of either sentinel or control houses, whereby the former are houses located within a village to be targeted with IRS but will not be sprayed; and the latter is to include houses in neighbouring villages which are not scheduled to be sprayed (World Health Organization, 2010). Here, sentinel houses are to demonstrate the mass effect of IRS on the vector population, whilst control houses when compared to sprayed houses; would allow for identification of seasonal or societal effects on sand fly densities, which may cause interference on the effect IRS has. Where insects can be dissected their parity can be determined, however determining parity in sand flies is problematic and therefore not routinely undertaken, and a larger emphasis is given to the sex and physiological status.

1.4.2.3 Vector susceptibility

In order to assess effectiveness of insecticides on the vector, the WHO guidelines advise annual susceptibility testing to be conducted following the WHO Tube test methodology (World Health Organization, 2015). Whilst the indicator is included within the Monitoring and Evaluation Tool kit for IRS, designed to support VL elimination in Bangladesh, Nepal and India, detailed guidance about monitoring the indicator only exists within WHO guidelines primarily designed for use by countries for mosquitoes that transmit malaria (World Health Organization, 2015). As a result, the methodology used for monitoring insecticide susceptibility has been directly transferred to sand flies within VL-endemic areas, and in the absence of appropriate sand fly populations to enable effective

discriminating dose calculations, concentrations developed for use when testing mosquitoes have been adopted. Discriminating doses are determined by establishing the 2x calculated lethal dose at which 99% of test vectors, mosquitoes, are killed (Lissenden *et al.*, 2021). The use of discriminating doses for mosquitoes on sandflies could have significant limitations for interpreting knock-down and mortality data generated for sand flies, as this does not factor any differences between the two diptera e.g. behaviour or size. Use of colony *P.argentipes* sand flies reared in laboratory environments, such as that available from Professor Petr Volf's laboratory (Lawyer *et al.*, 2017) could support with understanding the true diagnostic dose for the VL vector. Furthermore, at the point when the Indian VL IRS programme opted to switch to alpha-cypermethrin, a WHO diagnostic dose against mosquitoes was not available to order through WHO channels.

1.4.3 Presence of parasites in the vector

Parasite detection in the vector is seen as a critical activity for malaria and lymphatic filariasis (LF) elimination (World Health Organization, 2002; Laney *et al.*, 2010; Kefi *et al.*, 2018) and is seen as a tool to determine the effectiveness of control measures and progresses made towards elimination goals. Common practices include dissection of mosquitoes, biochemical assays and molecular analysis using polymerase chain reaction to identify infectious and infective stages of the parasite in various parts of the insect's body.

As an example, in malaria, salivary glands are dissected for microscopic examinations, however these methods are dependent on the availability of experienced personnel and are prone to sensitivity and specificity issues related to the hemolymph stage parasite contamination in the specimens (Kefi *et al.*, 2018). Alternatives such as polymerase chain reaction are highly sensitive and allow for higher throughput analysis, however, require specialist equipment and reagents, and skilled staff. For LF, determining the number of third stage filaria larvae, which individuals could be exposed to through infective vectors is essential for determining the transmission potential (World Health Organization, 2002).

Similarly molecular methods have been developed and implemented to determine the prevalence of *L. donovani* infection in *P. argentipes* sand flies in the VL endemic state of Bihar, India (Tiwary *et al.*, 2012; Cameron *et al.*, 2016).

1.5 Aims and Objectives

AIM: To investigate the hurdles in achieving effective indoor residual spraying for visceral leishmaniasis elimination in India

- Review historical datasets to critically assess previous achievements in VL elimination in India

- Determine the viability of post-IRS insecticide quantification methods to determine IRS quality
- Measure entomological and epidemiological indicators from 2016-2019 to determine progresses made in achieving VL elimination in India.

2 Visceral leishmaniasis cyclical trends in Bihar, India – implications for the elimination programme.

This chapter has been published in Gates Open Research, reference below:

Deb RM, Stanton MC, Foster GM, Das Gupta RK, Roy N, Das P, Dhariwal AC, Coleman M. Visceral leishmaniasis cyclical trends in Bihar, India - implications for the elimination programme. Gates Open Res. 2018 Feb 21;2:10. doi: 10.12688/gatesopenres.12793.1. PMID: 30234191; PMCID: PMC6139379.

Contributions: Rinki M Deb was responsible for study data collection, analysis, interpretation and for writing the manuscript for the above referenced paper. Statistical support was provided by Dr Michelle Stanton. Dr Michael Coleman and Dr Geraldine Foster assisted with design. Dr Das Gupta, Dr Dhariwal and Dr Roy also supported with design.

2.1 INTRODUCTION

Visceral leishmaniasis (VL) is a tropical disease of public health importance in India. Caused by *Leishmania donovani* parasites and transmitted by the sand fly *Phlebotomus argentipes*, the disease is anthroponotic in India and endemic in four States: Bihar, Jharkhand, West Bengal and Uttar Pradesh (Addy & Nandy, 1992). It is estimated that 90% of VL cases in India originate from Bihar (Singh *et al.*, 2010). In 2005, India with Bangladesh and Nepal, set a target to eliminate VL with incidence at less than one case per 10,000 population by 2015, which was subsequently adjusted to 2017. This was in line with the London declaration on Neglected Tropical Diseases (NTDs), which set the aim to eliminate VL as a public health problem by 2020.

The seasonal and interannual scale climate trends affecting vector-transmitted diseases have been widely reported (Connor, Thomson and Molyneux, 1999). In India, malaria prediction was first conducted using climatic and socioeconomic data in the 1910s by Captain S. R. Christophers from the British Army (Gill, 1938). This system was implemented until the 1940s, when malaria was not seen as disease of public health concern within the Indian subcontinent (Gill, 1938). Despite the burden of VL disease in India, little work has been done to associate climatic indicators with case incidence. Historical trends have previously shown that there is a resurgence of VL every 15 years post control: whilst widely accepted, there is limited understanding about the cause of this pattern (Malaviya *et al.*, 2011; Muniaraj, 2014).

Napier first proposed that VL in India was cyclical in nature in 1946, before the epidemics of 1977 and 1991. Visceral leishmaniasis epidemics in Assam (1875 and 1950) however were thought to be caused by the influenza pandemic of 1918–1919, malaria, famine and earthquakes (Rogers, 1908; McCombie Young, 1924; Napier, 1943). Findings from McCombie Young (1924) in Assam, supported this and after the introduction of antimonial drugs, a dramatic decrease in case mortality from 90% to 10% was observed (McCombie Young, 1924; Dye, 1992; Muniaraj, 2014). More recently, Muniaraj attempted

to elucidate the cause behind the 10–15 year cycle, attributing the VL case trend to vector control practices and the need for appropriate therapeutic strategies to reduce VL mortality (Muniaraj, 2014). However, Dye has referred to this cyclical trend of *L. donovani* VL as "part of the folk wisdom of tropical medicine", suggesting that post-kala-azar dermal leishmaniasis (PKDL) patients serve as a parasite reservoir: chronically infective and available to sand flies (Rogers, 1908; Dye, 1992). Studies by Addy and Nandy have suggested that disease transmission during the suspected epidemic period of 1977 in Bihar, was most likely passed on person to person from active VL cases (Addy and Nandy, 1992). Associations between climatic indicators, vector density and VL incidence in India have previously been detected when analysing district level data (Bern, Courtenay and Alvar, 2010; Malaviya *et al.*, 2011; Tiwary *et al.*, 2013; Dhimal, Ahrens and Kuch, 2015). Generalised associations for Bihar as a State, currently do not exist. Humidity, temperature, rainfall, soil temperature and moisture are all widely accepted factors that influence the development of *P. argentipes* (Bhunia *et al.*, 2010). There are two seasonal peaks of *P. Argentipes* in Bihar, one from March to June and another in October to November (Dinesh *et al.*, 2001; Picado *et al.*, 2010).

Associations have also been made between VL incidence and air temperature, relative humidity and annual rainfall in the Gangetic plain, which includes regions within Bihar (Bhunia *et al.*, 2010). Malaviya *et al.* noted seasonality of VL case trends, with a peak from March to April and a minor secondary peak in July (Malaviya *et al.*, 2011). In addition, the onset of disease symptoms were recorded, in descending order, from April to June, June to September and October to December (Perry *et al.*, 2013). This chapter investigates the impact of annual climatic and disease trends on the cyclic nature of VL burden in Bihar.

2.2 METHODS

2.2.1 Study area

The State of Bihar is located in the eastern part of India, extending over 94,163 km^2 and averages 52.73m above sea level. It is entirely land-locked, bounded by Nepal in the north and the State of Jharkhand in the south. To the east lies the humid State of West Bengal and to the west, the sub-humid State of Uttar Pradesh. There are 38 districts in Bihar and the total population in the 2011 census, was 104,099,452.

2.2.1.1 Data sources

Data sources can be seen in Table 1:

Table 1: Climate and VL case data sources(Deb et al.*, 2018)*

Data	Data Origin	Source	Accessed Date
Case Data	National Vector Borne Diseases Control Programme, Ministry of Health and Family Welfare	Kala-azar Cases and Deaths in the Country since 2010 (http://nvbdcp.gov.in/ka-cd.html)	30 June 2016
		Kala-azar Cases and Deaths in the Country since 2007 (http://nvbdcp.gov.in/ka-cd.html)	08 December 2014
		Sanyal RK, Banerjee DP, Ghosh TK *et al.* A longitudinal review of kala-azar in Bihar. *J Commun Dis* 1979;11:149–69.	Published article
		Bora D. Epidemiology of visceral leishmaniasis in India. *Natl Med J India* 1999;12:62–8.	Published article
		Thakur CP, Kumar A, Mitra G *et al.* Impact of amphotericin-B in the treatment of kala-azar on the incidence of PKDL in Bihar, India. *Indian J Med Res* 2008;128:38–44.	Published article
Rainfall	Indian Institute of Tropical Meteorology	ftp://www.tropmet.res.in/pub/data/rain/iitm-subdivrf.txt	08 December 2014
	Earth System Science Organisation (ESSO)-India Meteorological Department (MoES)	http://www.imd.gov.in/press_release/20160602_pr_31.pdf	08 December 2016
Temperature and Humidity	International Research Institute for Climate and Society	http://iridl.ldeo.columbia.edu/	08 June 2016
	National Centers for Environmental Prediction (NCEP) and the National Centre for Atmospheric Research (NCAR) as part of the NCEP/NCAR Climate Data Assimilation System (CDAS) Reanalysis Project	http://www.cpc.ncep.noaa.gov/products/wesley/reanalysis.html	08 June 2016
Climate Periodicity	Published literature	Dinesh DS, Ranjan A, Palit A *et al.* Seasonal and nocturnal landing/biting behaviour for *Phlebotomus argentipes* (Diptera: Psychodidae). *Ann Trop Med Parasitol* 2001;95:197–202.	Published article

For Temperature and humidity, the spatial resolution is 1 degree (according to the text) which is equivalent to around 111km at the equator. Rainfall data is derived from merged satellite and weather station data.

2.2.1.2 Epidemiological data

Yearly case numbers from Bihar were compiled using open access data available from the National Vector Borne Disease Control Programme (NVBDCP), Government of India (Directorate National Vector Borne Disease Control Programme, 2016a, n.d.a, n.d.b) and published literature quoting historical case data from Government of Bihar (GOB) and Government of India records. Search term strings used to identify historical case data for Bihar included “visceral leishmaniasis”, “kala-azar”, “Bihar”, “case” and “incidence” on Pubmed and Google Search engines. Case numbers obtained from the NVBDCP originate from primary health care facilities and hospital records within Bihar, where

diagnostic testing, followed national guidelines (Directorate National Vector Borne Disease Control Programme, n.d). Annual incidence was calculated using population totals obtained from Bihar population data, obtained from decennial census surveys conducted by Ministry of Home Affairs, Government of India (Directorate of Economics & Statistics (Bihar Patna), 2011). Annual population growth statistics from the Directorate of Economics and Statistics, Patna, Bihar (1956–1959) and the World Bank Databank (1961–2014) was used to produce yearly population figures between census surveys (by multiplication of annual growth rate and population for previous year). Census numbers for Bihar were taken directly where available. Visceral leishmaniasis incidence was calculated per 10,000 for all years where case and total population data were available.

2.2.1.3 Indoor residual spraying (IRS) data

Peer-reviewed literature searches were used to determine years when IRS was performed in Bihar between 1931–2014 (Date of last search: 31 November 2015). The search term strings included "visceral leishmaniasis", "kala-azar", "indoor residual spraying", "IRS", "India" and "Bihar" were used on PubMed and Google search engines to establish historical spray activities. Bibliographies from relevant peer-reviewed journals were used to identify additional information and data.

2.2.1.4 Rainfall data

Bihar State historical monthly rainfall data (1871–2013) was obtained at the time of analysis from the Indian Institute of Tropical Meteorology. Adapted Earth System Science Organisation (ESSO)-India Meteorological Department (MoES) long period average (LPA) rain fall categories were used to classify rainfall data for the selected time period into standardised groups: Deficient = rainfall below 80% LPA, Below Normal = rainfall 80–90% LPA, Normal = rainfall 90–110% LPA, Above Normal = rainfall 110–120% LPA and Excess = rainfall above 120%.

2.2.1.5 Temperature and humidity

The average and maximum temperature and specific humidity data corresponding to the coordinates of Bihar were extracted from satellite sources and digital databases through the International Research Institute for Climate and Society website. Data for Bihar, accessed through the website, originated from the National Centers for Environmental Prediction (NCEP) and the National Centre for Atmospheric Research (NCAR), as part of the NCEP/NCAR Climate Data Assimilation System (CDAS) Reanalysis Project. Daily data provided at a 1 degree spatial resolution were aggregated to provide a monthly output for the entire State. Temperature data was converted from Kelvin to degrees Celsius.

2.2.1.6 Climate periodicity

In order to aggregate monthly climate data into an appropriate annual indicator, literature searches were conducted, and two clear time periods were identified as the most relevant for VL transmission:

Monsoon Season (June-September), and Sand Fly Peak abundance (spanning months March – June and October – November) (Dinesh et al., 2001).

As the sand fly life cycle is estimated to take approximately one month, a one-month window prior to sand fly abundance peaks (spanning months February-May and September-October), categorised as Pre-Sand Fly Peak, was also considered relevant in determining risk factors associated with VL transmission.

Finally, the Annual category for all variables was used to identify any trends between annual VL incidences and calendar year annual climatic variables.

2.2.1.7 Analysis

Monthly data from the specific climatic periods (Annual, Monsoon Season, Sand Fly Peak and Pre-Sand Fly Peak) were averaged to produce a single annual figure per time period. A non-parametric Kruskal-Wallis Test was performed to determine whether VL incidence varied between the different annual rain categories (deficient, below normal, normal, above normal, excess). Univariate negative binomial regression models were fitted to the VL incidence data for each of the climatic indicators (specific humidity, average and maximum temperature) using data from the periods 1956–1960 and 1977–2013. To account for multiple testing, the resulting p-values were adjusted using the false discovery rate (FDR) correction (Benjamini & Hochberg, 1995). Climate variables with a p-value less than 5% were significant, plus the Akaike Information Criterion (AIC) values were compared to determine which of the models was the best fit to the data. To account for long-term changes in climate and VL incidence, the analysis was repeated using temporally continuous data only (1977–2013) using climate anomalies (observed values – period average) and a linear temporal trend as predictors in the negative binomial regression models. Analysis was conducted in R 3.2.5, SPSS (Version 22) and Graphpad Prism (Version 6.07).

2.3 RESULTS

2.3.1 Epidemiological data

A total of four peer-reviewed published papers were identified to provide data on historical VL case numbers over the analysis period. After analysis of the data all papers were included. The earliest record of case data available for Bihar was in 1934, however review of the historical literature also showed a disparity in the total number of cases reported between 1934 and 2014. A total of 1,190,166 cases were recorded when using GOB only records (Sanyal *et al.*, 1979; Directorate National Vector Borne Disease Control Programme, n.d.a, n.d.b). Conversely, 1,461,963 cases were recorded when including GOB records and independent surveys (GOB&I) data conducted through National Institute

of Communicable Diseases, Delhi (1977), and Government of India under the Malaria Department (1991).

Records of VL cases were unavailable between 1938–1955 and 1961–1976, most likely coinciding with public health priorities moving away from VL in Bihar. However, case records were available consistently from 1977 to present (Figure 6). As noted by Bora, "due to limited surveillance strategies" information on outbreaks before 1977–78 was patchy (Bora, 1999). Cases recorded were largely confirmed by tissue specimen microscopy, however since 2005 the NVBDCP guidelines advise use of the rk39 rapid diagnostic test. Other methods such as direct agglutination test, enzyme-linked immunosorbent assay, parasite culture and PCR may have been used at a smaller scale to diagnose cases presented in health facilities associated with research institutes.

GOB VL incidence data, collected solely through Primary Health Centres, showed the highest VL incidence was in 1992 (11.480 per 10,000 (75,523)) (Figure 6 & Table 3). The second highest VL incidence was recorded in 1991 (59614 cases (9.238 per 10,000). Bora reported that a further peak in VL cases occurred in 1974, however, as the programme assumed elimination had been achieved and stopped VL case data collection, no information was available to calculate incidence (Bora, 1999). The lowest incidences recorded were seen in 2014 (7615 cases – 0.705 per 10,000) and 2013 (1.005 per 10,000 (10730 cases) (Figure 6 & Table 3).

According to GOB&I data, the highest annual case reports were recorded in 1977 (100,000 cases – incidence of 20.720 per 10,000) and 1991 (250,000 cases – incidence of 38.741 per 10,000) (Figure 6). The large differences in VL incidence reported between datasets could be associated with the source of the dataset; with GOB data generated from passive data recording from government health facilities and hospitals, whilst the independent survey data would capture case numbers after active case searching efforts.

GOB data only was used in the analysis of climatic indicators.

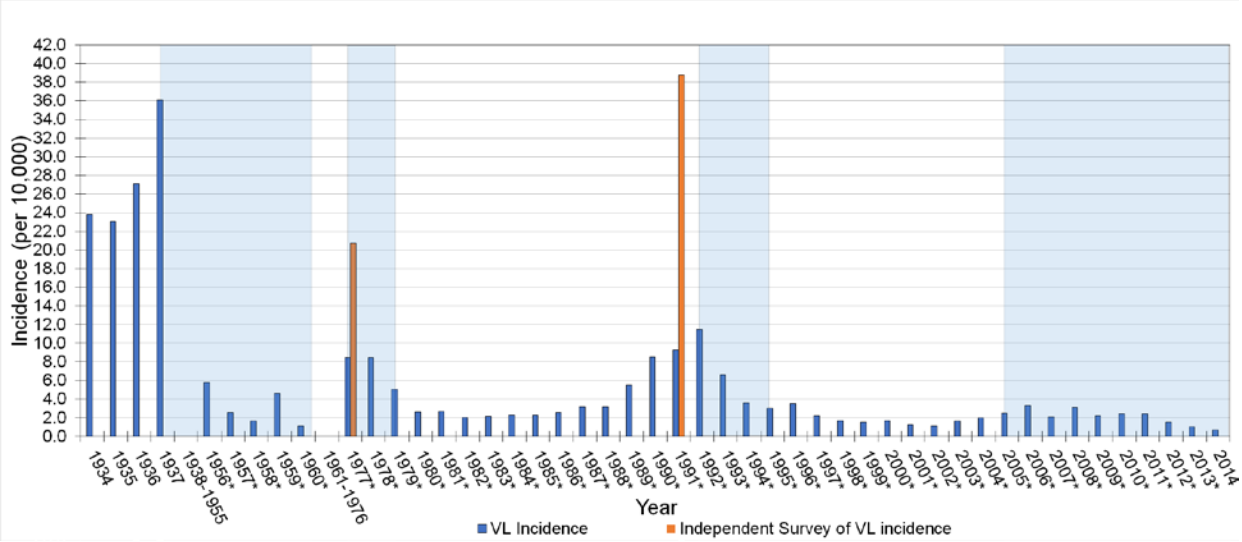

Figure 6: Historical Overview: Annual VL Incidence in Bihar and IRS activities (Deb et al.*, 2018)*

The dark blue bars represent the annual VL incidence per 10,000, as reported by the VL Programme (GOB only). In the absence of historical population at risk data, the total population in Bihar was used to calculate incidence. Independent surveys conducted in 1977 by National Institute of Communicable Diseases, Delhi and GOB and 1991 by Government of India under the Malaria Department, reported VL incidence per 10,000 is shown in orange for those two years (GOB&I). Light blue shading represents years where IRS was ongoing, whilst white represents years where IRS was stopped. Years marked with an asterisk were used in analysis of climatic, case and IRS indicators.

2.3.2 IRS data

Using the search terms, a total of four papers were identified to provide information on historical IRS activities in the region, after review no papers were removed from the analysis. IRS was first adopted for VL elimination in Bihar through the National Malaria Control Programme (NMCP) and the National Malaria Eradication Programme (NMEP) in the 1940s (Thakur, 1984). Activities were stopped from 1962–76 in response to a rapid fall in VL case numbers, however resumed for two years again in 1977, with support from Government of India, to combat the outbreak declared that year (Thakur, 1984; Muniaraj, 2014). Following a survey in 1991, an outbreak of VL was declared and IRS was conducted 1992–1995, after which IRS was stopped (Thakur, 2007). In 2005, with the launch of the "Kala-azar Elimination Programme", IRS was restarted to reach the elimination target (less than one in 10,000 within a primary healthcare centre/block) by a recently revised deadline of 2020 (Mondal *et al.*, 2009b; World Health Organization, 2016c). No records of IRS activities were available during years 1934–1936, when there was a high case burden, or for the VL peak of 1974 (Bora, 1999).

Table 2: Overview of IRS activities for VL in Bihar, India (Deb et al.*, 2018)*

IRS	Years	Programme Name	Programme led by
1	1937-1962	National Malaria Eradication Programme	National-run Programme
2	1977-1979	Kala-azar Control Programme	National Institute of Communicable Diseases with UNDP assistance
3	1992-1995	Kala-azar Control Programme	
4	2005-Present	Kala-azar Elimination Programme	State-run Programme

2.3.3 Rainfall

Sixty-two years of rainfall data, 1951–2013, were available to determine trends and association between rain time categories and VL incidence, after accounting for the gaps in VL incidence data (1951–1955 and 1961–1976), a total of 42 matched years (1956–1960 and 1977–2013) were used to complete the analysis (marked with an * in Figure 6). The average (Annual) rainfall for the total 42 matched years was 1017.66mm. The average rainfall during Monsoon Season periods for the same matched years was 2517.92mm. When considering the sand fly-related time categories for these years, the average rainfall during Sand Fly Peak months was 586.46mm and 659.01mm during the Pre-Sand Fly Peak months.

After categorising the data into the five standardised groups, the rainfall during Annual and Monsoon Season time periods were found to be mostly Normal to the LPA (18/42 (42.86%) and 14/42 (33.33%) respectively). During the Sand Fly Peak period, rainfall was classified most frequently as Deficient to the LPA (13/42 (30.91%)). Conversely, when considering the Pre-Sand Fly Peak period, rainfall was found to be Excess to the LPA most frequently (12/42 (28.57%)).

2.3.4 Temperature and humidity

A total of 66 years of temperature and humidity data was available from data sources, however, given the limited availability of VL incidence data, a total of 43 years of temperature and specific humidity data (1956–1960 and 1977–2014) were used. As shown in Table 3, the mean average temperature over the different time groupings ranged between 21.74 and 25.36°C whilst the mean maximum temperature was 28.36–31.14°C. The mean specific humidity (x1000) ranged between 12.000 – 20.000kg/kg.

Table 3: Temperature and Humidity descriptive for all time periods (Deb et al.*, 2018)*

		Annual	Monsoon Season	Sand Fly Peak	Pre-Sandfly Peak
Average Temperature	Mean (°C)	21.74	25.36	23.73	22.99
	Range (°C)	20.88-23.25	24.05-26.77	22.85-25.78	21.95-24.60
	s.d.	0.551	0.446	0.684	0.782
	Coefficient	0.6818	0.8708	0.8383	0.7959
	95% CI	0.5040 – 0.9355	0.5127 – 1.4919	0.6541 – 1.0914	0.6454 – 0.9890
	p-value	0.0186*	0.5142	0.1788	0.0494*
	Adjusted p-value	0.1116	0.6856	0.3576	0.1503
	AIC	927	932	931	929
Maximum Temperature	Mean (°C)	28.36	28.75	31.14	30.18
	Range (°C)	27.30-29.75	27.21-30.81	29.89-32.84	28.78-31.97
	s.d.	0.645	0.645	0.796	0.866
	Coefficient	0.667	0.9466	0.865	0.8137
	95% CI	0.5055 – 0.8863	0.6656 – 1.3574	0.6793 – 1.1086	0.6660 – 0.9977
	p-value	0.003*	0.709	0.2174	0.0501¤
	Adjusted p-value	0.0360*	0.8508	0.3727	0.1503
	AIC	926	933	931	929
Specific Humidity (x1000)	Mean (kg/kg)	13	13	13	13
	Range (kg/kg)	12.000 – 13.000	18.800 – 20.000	11.000 – 13.000	10.000 – 13.000
	s.d.	0.00033	0.00031	0.00056	0.00053
	Coefficient	0.6664	0.9664	0.9845	0.8296
	95% CI	0.4121 – 1.0313	0.6600 – 1.4013	0.7431 – 1.3011	0.5784 – 1.1716
	p-value	0.0813	0.8586	0.9165	0.2811
	Adjusted p-value	0.1951	0.9165	0.1965	0.4217
	AIC	930	933	933	932

Negative Binomial model with VL incidence using data from GoB only. * P-value is significant at the 0.05 level. ¤P-value is nearly significant. (Akaike Information Criterion (AIC), Confidence interval (CI), standard deviation (s.d.))

2.3.5 Climatic variables and VL incidence

Rainfall during 1992, the highest VL incidence according to GOB data, was classified as Deficient during all time periods (Table 4). During the year of the second highest VL incidence, rainfall was classified as Below Normal to the LPA when considering the Annual and Monsoon Season time periods (Table 4). During Sand Fly Peak and Pre-Sand Fly Peak time periods, rainfall was Deficient to the LPA.

The lowest VL incidence was recorded in 2014, however no rainfall data was available for this time frame. VL incidence was recorded as low in 2013 (1.005 per 10,000) during which, rainfall was classified as Deficient during the Monsoon Season, Excess during the Pre-Sand Fly Peak and Sand Fly Peak, and Normal for the Annual (Table 5).

When considering VL incidence and rainfall together, no statistically significant relationship was detected for any of the time periods (Annual (p=0.265), Monsoon Season (p=0.281), Sand Fly Peaks (p=0.602) and Pre-Sand Fly Peak (p=0.416)) (Table 5). Overall, average VL case incidence, irrespective

of time period, was lower (2.172–3.133) during years of higher rainfall (Above Normal or Excess to the LPA).

The average VL incidence when considering all 22 IRS years, between 1956 and 2014 (Table 2), was 3.590. For the 21 years when IRS stopped, the average VL incidence was 3.000. The negative binomial regression model fitting identified statistically significant negative associations between both *Average Temperature* and *Maximum Temperature* and VL incidence (GOB data) during the *Annual* (RR= 0.682, p=0.0186 and RR=0.667, p=0.003 respectively) and *Pre-Sand fly Peak* (RR=0.796, p=0.0494 and RR= 0.814, p=0.0501 (close to statistically significant) respectively) time periods only (Table 3). However, after correcting for multiple testing using the FDR correction, only Annual Maximum temperature was significant (p=0.0360). Of these regression models, Annual Maximum temperature was the best fitting model as assessed using AIC. No other significant associations were detected for temperature and other time periods, or humidity and VL incidence (Table 3). Summaries of the models fitted to climate anomalies for the period 1977–2013 can be found in Table 6. After adjusting for a decreasing temporal trend in incidence, only annual specific humidity anomalies were associated with incidence (p=0.0398) however after adjusting for multiple testing this association became non-significant (p=0.4776).

Table 4: Rainfall, VL case incidence per 10,000 (GOB data) and Kruskal-Wallis analysis (Deb et al.*, 2018)*

Year	VL incidence (per 10,000)	Annual				Monsoon Season				Sand Fly Peaks				Pre-Sand fly Peaks			
		Ave. Temp (°C)	**Max. Temp (°C)**	**Specific Hum. (kg/kg)**	**Rainfall**	**Ave. Temp (°C)**	**Max. Temp (°C)**	**Specific Hum. (kg/kg)**	**Rainfall**	**Ave. Temp (°C)**	**Max. Temp (°C)**	**Specific Hum. (kg/kg)**	**Rainfall**	**Ave. Temp (°C)**	**Max. Temp (°C)**	**Specific Hum. (kg/kg)**	**Rainfall**
1991	9.238	21.64	28.34	0.013	Below Normal	25.64	29.09	0.020	Below Normal	23.62	31.47	0.012	Deficient	23.05	30.59	0.012	Deficient
1992	11.480	21.12	27.97	0.012	Deficient	25.39	29.23	0.019	Deficient	23.44	31.29	0.011	Deficient	22.09	29.75	0.011	Deficient
2013	1.005	22.38	29.38	0.013	Normal	22.38	28.93	0.020	Deficient	24.63	32.31	0.012	Excess	24.42	31.92	0.012	Excess
2014	0.705	22.46	29.75	0.013	N/A	22.46	30.81	0.020	N/A	24.56	32.83	0.011	N/A	23.55	31.17	0.012	N/A

(Average temperature (Ave. Temp), Maximum temperature (Max. Temp), Specific Humidity (Specific Hum.))

Table 5: Annual and Maximum Temperature, Humidity and Rainfall during high and low VL incidence (Deb et al.*, 2018)*

		Annual						Monsoon						Sand fly Peaks						Pre Sand fly Peaks					
Rainfall Category	**Description**	Rainfall Range/ mm	# Years	Incidence per 10,000			P value	Rainfall Range/ mm	# Years	Incidence per 10,000			P value	Rainfall Range/ mm	# Years	Incidence per 10,000			P value	Rainfall Range/ mm	# Years	Incidence per 10,000			P value
				Min.	Max.	Ave.				Min.	Max.	Ave.				Min.	Max.	Ave.				Min.	Max.	Ave.	
Deficient	**<80% LPA**	670.250-792.250	7	1.148	11.480	3.305		1663.750-2010.250	9	1.005	11.480	3.291		255.333-454.167	13	1.148	11.480	3.704		265.000-524.166	9	1.702	11.480	4.462	
Below Normal	**80-90% LPA**	825.333-887.500	5	1.702	9.238	4.351		2038.750-2237.250	7	1.233	9.238	3.492		480.833-524.167	6	1.698	8.523	3.489		532.166-574.000	9	1.148	8.523	3.193	
Normal	**90-110% LPA**	926.833-1089.916	18	1.005	8.523	3.700	0.265	2340.500-2764.500	14	1.181	8.523	4.034	0.281	543.500-627.833	7	2.056	6.587	3.672	0.602	598.833-720.000	7	2.140	4.798	2.961	0.416
Above Normal	**110-120% LPA**	1124.333-1198.585	6	1.630	5.967	2.889		2780.750-2848.750	5	1.623	5.967	3.033		660.833-697.333	6	1.991	4.798	2.970		737.000-781.833	5	1.560	6.587	2.923	
Excess	**>120% LPA**	1253.750-1441.083	6	1.560	3.982	2.172		3085.000-3960.750	7	1.560	3.182	2.304		723.000-960.667	10	1.005	8.485	2.924		792.833-1029.333	11	1.005	8.485	3.133	

VL incidence based on data from GOB data. (Minimum (Min.), Maximum (Max.), Average (Ave.)

Table 6 Summaries of the models fitted to climate anomalies for the period 1977-2013. (Deb et al., 2018)

		Annual	Monsoon Season	Sand Fly Peak	Pre-Sandfly Peak
Average Temperature	Mean (°C)	21.73	25.36	23.77	22.99
	Range (°C)	20.88-23.25	24.05-26.77	22.85-25.78	21.95-24.6
	s.d.	0.551	0.446	0.710	0.782
	Climate Anomaly Coefficient	0.6783	0.9965	0.8004	0.8014
	95% CI	0.3969-1.1503	0.6306-1.5864	0.5666-1.157	0.5634-1.1437
	p-value	0.1493	0.9855	0.2055	0.2456
	Adjusted p-value	0.6290	0.9855	0.6290	0.6290
	Temporal trend	0.9675	0.9669	0.9672	0.9674
	95% CI	0.9514, 0.9838	0.9501, 0.9843	0.9510, 0.9836	0.9512, 0.9840
	p-value	<0.0001	<0.0001	<0.0001	<0.0001
	Adjusted p-value	<0.0001	<0.0001	<0.0001	<0.0001
	AIC	800	802	800	800
Maximum Temperature	Mean (°C)	28.36	28.75	31.14	30.18
	Range (°C)	27.3-29.75	27.21-30.81	29.89-32.84	28.78-31.97
	s.d.	0.645	0.644	0.795	0.866
	Climate Anomaly Coefficient	0.7942	1.0378	0.9685	0.8994
	95% CI	0.5254-1.2025	0.7666-1.4157	0.7348-1.2831	0.6816-1.187
	p-value	0.2621	0.7831	0.8243	0.4762
	Adjusted p-value	0.6290	0.9855	0.9855	0.9524
	Temporal trend	0.9670	0.9672	0.9668	0.9671
	95% CI	0.9507, 0.9836	0.9503, 0.9846	0.9500, 0.9840	0.9505, 0.9840
	p-value	<0.0001	<0.0001	<0.0001	<0.0001
	Adjusted p-value	<0.0001	<0.0001	<0.0001	<0.0001
	AIC	801	802	802	801
Specific Humidity (x1000)	Mean (kg/kg)	12.81	19.60	12.14	11.60
	Range (kg/kg)	12-13	19-20	11-13	10-12
	s.d.	0.394	0.495	0.639	0.541
	Climate Anomaly Coefficient	0.5984	1.0085	0.9242	0.9105
	95% CI	0.3542-0.9582	0.6802-1.4663	0.7073-1.2042	0.6082-1.3442
	p-value	0.0398*	0.9652	0.5980	0.6353
	Adjusted p-value	0.4776	0.9855	0.9530	0.9530
	Temporal trend	0.9673	0.9670	0.9673	0.9672
	95% CI	0.9515, 0.9835	0.9501, 0.9841	0.9505, 0.9845	0.9503, 0.9843
	p-value	<0.0001	<0.0001	<0.0001	<0.0001
	Adjusted p-value	<0.0001	<0.0001	<0.0001	<0.0001
	AIC	798	802	802	802

* P-value is significant at the 0.05 level

2.4 DISCUSSION

Associations between climatic indicators and vector-borne diseases have been established within many diseases, including VL (Thomson *et al.*, 1999, 2005; Malaviya *et al.*, 2011; Chen *et al.*, 2012; Perry *et al.*, 2013; Tiwary *et al.*, 2013; Dhimal, Ahrens and Kuch, 2015). Predictors of Leishmaniasis spp. Transmission cycles and sensitivity to meteorological and climatic variables is known to vary spatially; dependent on a range of factors including species composition, host competence, contact rates, vector competence, sensitivity to weather and other environmental stressors (Chaves *et al.*, 2008; Lewnard *et al.*, 2014). These factors are essential for developing early warning systems to prevent epidemics. Due to the lack of spatial and temporal open access granular case data, there are a limited number of studies investigating the interaction of climatic variables and VL in Bihar. Previous studies have predominantly focused on district level climatic trends identifying various monthly or annual temperature, relative humidity and rainfall measurements as the key variables affecting VL transmission (Napier, 1926; Sivaramakrishnaiah and Ramanatham, 1967; Malaviya *et al.*, 2011; Tiwary *et al.*, 2013). This is the first study to look at historical data spanning 43 years and include climatic variables during specific month periods estimated to impact transmission, namely seasonal sand fly abundance and monsoon period (Picado *et al.*, 2010).

In this study, four statistically significant negative associations were detected between Average Temperature and VL incidence (RR= 0.682, p=0.0186 (Annual) and RR=0.796, p=0.0494 (Pre-Sand fly Peak)) and Maximum Temperature and VL incidence (RR=0.667, p=0.003 (Annual) and RR= 0.814, p=0.0501 (near significant – Pre-Sand fly Peak), using GOB data (Table 2). However, after considering the FD correction for multiple testing, only Maximum Temperature and VL incidence retained a significant association (0.0360). Overall, this shows that transmission risk is reduced with each unit increase of temperature. This could be due to human behavioural patterns, such as more people sleeping outside in warmer temperatures, unfavourable temperatures for optimal sand fly emergence or other trends associated with the vector and transmission dynamics. This is the first time that an association with temperature has been suggested to VL incidence for the Pre-Sand fly Peak time-period. Annual humidity and VL incidence when fitted to climate anomalies (1977–2013) and adjusted for a decreasing temporal trend in incidence, also showed some association (p=0.0398) however only when corrections for multiple testing were not considered. This suggests that Annual Humidity could also play a similar key role in VL transmission to Annual Maximum Temperature: influencing human sleeping behaviour.

Historical research indicated that the factors favouring the development of an epidemic would include altitude below 609.60m above sea level, greater than 1270mm annual rainfall, greater than 70% mean humidity, presence of alluvial soil, maximum temperature below 37.78°C, with diurnal variation less

than – 6.666°C, abundant vegetation and subsoil water, and a rural environment (Napier, 1926; Sivaramakrishnaiah & Ramanatham, 1967). Bihar is estimated to be 52.73m above sea level, and maximum temperature was below 37.78°C across all time periods (Table 3), however rainfall during years of high VL incidence was considerably lower than previously suggested (Table 5). Other criteria such as "abundant vegetation and subsoil water" cannot be quantified to form a comparable variable within this study. Given the changes in global climate, these historical guidelines on optimal conditions for a VL epidemic may no longer be suitable.

More recent research conducted by Malaviya *et al.* (Malaviya *et al.*, 2011) showed associations between monthly VL incidence, rainfall and specific humidity, but contrary to this study, found no clear association between incidence and average or maximum temperature. A study conducted in the Gangetic plain, which includes VL endemic West Bengal and Bangladesh, suggested optimal meteorological factors for VL incidence included air temperature of 25.0–27.5°C, relative humidity of 66–75% and annual rainfall between 1000 and 1600 mm (Bhunia *et al.*, 2010). Due to a lack of available open access data, only annual rainfall data can be compared to findings by Bhunia *et al.* (Bhunia *et al.*, 2010): rainfall during the periods of high VL incidence fell below the suggested optimal range (1992: 834.667mm, 1991: 670.250mm).

The term epidemic is generally referred to as a sudden increase in the number of cases, beyond what is normally expected within the population of a specific area (Centers for Disease Control and Prevention (CDC), no date). The underlying assumption when identifying an epidemic is that the data collection methods adopted are comparable in accuracy and precision. When including GOB&I data, the highest VL incidences were in 1977 and 1991, and historically they have been referred to as "epidemics" (Thakur, 1984, 2007; Bora, 1999; Thakur *et al.*, 2008). Data forming the basis for these suspected epidemics were collected through active case detection surveys. Such surveys typically detect higher levels of incidence than those recorded through normal passive reporting channels and highlight the need for better case reporting mechanisms. Comparisons between GOB and GOB&I data suggest that case numbers recorded through standard reporting channels in 1977 and 1991 were under-reported (81.41% and 76.15% respectively). Under-reporting of VL in Bihar has been documented since 1977, with most recent reports up to 2006 (Thakur, 1984; Bora *et al.*, 1994; Singh *et al.*, 2010). In 2010, it was reported that a "substantial proportion" of patients were visiting private laboratories for diagnosis, which could explain underreporting documented for VL (Hasker *et al.*, 2010). The quality of passive case reporting may have fluctuated greatly over the years, so a single correction factor cannot be applied across all years of data without further evidence. While it is still questionable that these were true epidemics, both surveys resulted in the implementation of IRS programmes to control the disease.

Patterns seen in Figure 6 suggest IRS and case numbers are tracking each other and is particularly prominent after the two peaks in 1977 and 1992. As the data is annual the timing of spray is not clearly visible, however this is most likely a reaction to the two independent surveys on 1977 and 1992; whereby the national programme restarted IRS activities after a significant increase in case numbers were reported. When considering the current data set, as the peaks originate from independent surveys, they may simply be artefacts that highlight the true burden of disease. As reported by Bora *et al.,* case data recorded by GOB typically only include cases recorded through government health facilities, as private practitioners treating for VL fail to report cases they have treated to the health authorities (Bora *et al.*, 1994). This was also the issue faced by Dye and Wolpert when modelling the Assam epidemics of 1875 and 1950; where poor quality data limited the findings for the cause behind the second epidemic (Dye and Wolpert, 1988). Given the discrepancies in data and sources, further evidence is required to confirm the presence of a VL disease cycle.

The anthroponotic nature of the disease in India, suggests records of PKDL could provide additional evidence to explain the peaks seen in 1977 and 1991 (Addy and Nandy, 1992). Open access case data sources used within this analysis, provided the number of deaths and cases only, restricting further exploration using this variable.

Historically VL vector monitoring within Bihar has been sporadic and limited by lack of spatial and temporal granularity (Dinesh *et al.*, 2001; Picado *et al.*, 2010; Malaviya *et al.*, 2011; Tiwary *et al.*, 2013). This has prevented the robust multiannual evaluation of *P. argentipes* abundance peaks, physiology status and transmission effectiveness. For effective retrospective analysis to understand disease incidence, understanding the vector is crucial, furthermore climatic parameters can be further refined based on vector behaviour. Time periods used within this study were identified as periods of high sand fly activity or adverse weather. The months included within the Sand Fly Peak time period (March – June and October – November) have been shown previously to coincide with months of high case numbers (Dinesh *et al.*, 2001). This relationship needs further exploration, through spatial models, if monthly granular data were available, to further understand disease trends and outbreak prediction.

The available published data describes Bihar's primary intervention against VL, IRS, as 42ichotomyous variable, limiting the understanding of its impact on the natural disease patterns. The VL incidence between IRS and non-IRS years is very similar when considering the overall dataset and interestingly increases (3.590) during years of IRS in comparison to years of non-IRS (3.000), suggesting that associations are potentially being masked by the lack of detail about the intervention. Other IRS programme indicators, such as spray quality, would also supplement understanding the success of IRS activity, allowing for a robust statistical model to be developed (Coleman *et al.*, 2015).

Since the start of the VL control efforts, strategies adopted for case finding have changed, with the most concerted efforts ongoing to support evaluation of progress towards the current elimination target. Accredited Social Health Activists (ASHAs) today play a key role in promoting healthcare activities such as immunisation, reproductive and child health and detection of VL cases (Das *et al.*, 2016). Research has shown that the incentive-based approach adopted in India for identifying VL patients led to an improved referral rate, translating into early detection of cases and a long-term drop in case numbers (Das *et al.*, 2016).

The Indian VL elimination programme has taken significant steps to improve case data quality over recent years: increasing VL awareness, providing incentives to local health workers (ASHAs) and implementing district level tracking systems. For the VL elimination programme this means that to sustain the gains made a system such as reactive IRS as used in some places for malaria must be established, or the levels of VL will increase again as observed in Figure 6 (Coleman *et al.*, 2008). Using the current WHO standards for initiating alerts in India, as used in Brazil, of consecutive months when the incidence has been double the monthly average, is likely to be a sub-optimal criterion with limited infrastructure and funding to ensure rapid response: particularly after the elimination target has been achieved and the disease is no longer considered a public health concern (Coleman *et al.*, 2008; Lewnard *et al.*, 2014). A comprehensive understanding of the causative factors for disease trends is required to develop a suitable outbreak detection and response system within the VL Indian context.

3 Field assessment of alternative sample collection methods for Indoor Residual Spraying Quality Assurance

Contributions:

Rinki was responsible for developing the study design, obtaining ethics approval, developing study protocols and for providing training to the team members on how to collect samples. Whilst field teams collected the samples, Rinki was responsible for supervising and leading the field teams, providing retraining, and for ensuring the protocols were adhered to throughout the field survey. Analysis of samples were done by laboratory-based staff, using a methodology developed and widely used within LSTM. Analysis and interpretations of results was done by Rinki Deb, with statistical guidance from LSTM statistician, Agnes Matope. Dr Mark Paine provided supervisory guidance on research design and results interpretation. Field teams involved in the sample collection were from Rajendra Memorial Research Institute, including Dr Rudra Pratap Singh who supported field implementation of the survey. Dr Pushkar Shivam supported with some HPLC analysis of samples collected from the field.

3.1 INTRODUCTION

Quality assurance of indoor residual spraying (IRS) activities, whereby the concentration of insecticide deposited on walls is measured, should be an essential component of IRS performance monitoring. The World Health Organization approved gold standard method for assessment, requires 5cm^2 Whatman Grade 1 filter papers to be affixed onto walls prior to IRS (World Health Organization, 2006a, 2010). Once sprayed and the filter paper is dry, the sample is collected and transported to a laboratory for high performance liquid chromatography (HPLC) analysis to provide a quantitative result (World Health Organization, 2006a).

The sample collection method is operationally problematic. There is inadequate guidance on how to attach the filter paper to different wall surfaces, such as brick. Once the filter paper is affixed to the wall, it is assumed that the paper will remain undisturbed until the point of collection, however papers could be removed by household members or detach from the wall. Repeated QA filter paper surveys can also lead to behavioural bias, as the papers are clearly visible and spray operators may target them, if they become aware that their performance is being assessed by this method (Russell *et al.*, 2014). Therefore, the results obtained from these filter papers may not be a true representation of the spray operator's overall performance.

Swabs (Morou *et al.*, 2008) or sticky-tape removals (Barlow, 1955; Russell *et al.*, 2014) have been trialled as an alternative method of extracting insecticide residues after a surface has been sprayed. Whilst some success with sticky-tape removals were reported when surveying wettable powder insecticides, such as DDT, which are poorly absorbent, both swabs and sticky tapes were found to have poor extraction efficiencies and had issues with surface variability (Barlow, 1955; Russell *et al.*, 2014).

In particular, the sampling efficiency of Sellotape was reported to be very low, with only ~10% of the applied insecticide extracted from sprayed surfaces (Dowd *et al.*, 2009). A study in Vanuatu compared pre-spray sampling with circular felt pads to post-spray sampling using Sellotape strips and the adhesive side of the felt pad. Here, the low sampling efficiency of Sellotape was demonstrated once more, however post-spray use of felt pads retrieved 60-80% of insecticide from surfaces, dependent on wall surface type (Russell *et al.*, 2014).

Cone bioassays using insecticide susceptible mosquitoes are the WHO gold standard entomological method to assess IRS efficacy and allow for continued assessment of the biological efficacy of the IRS over time through repeated testing (Silver, 2008). The method determines if the vector control strategy provides effective protection to the homeowner beyond the point of implementation, and therefore forms an essential component of continued IRS quality assurance monitoring. However, cone bioassays require readily available fully susceptible vectors, reared in an established insectary and available in high numbers, for testing to be conducted by specialist staff with entomological training. This method does not provide quantitative data on the dose of insecticide to which the vector has been exposed (Silver, 2008).

Effective IRS quality monitoring is essential to ensure stewardship of available insecticide products and to avoid the selection of operationally relevant levels of insecticide resistance through prolonged sub-lethal dosing. A better method of measuring the dose of insecticide applied and its rate of decay over time, which does not have the major issues of the current gold standards is needed. This chapter will consider potential alternative pre- and post- spray insecticide sample collection methods and assess if they might improve on the current WHO gold standard method. This is particularly important for the VL programme, as there are no available colonised insecticide susceptible sandflies in India to support cone bioassay QA.

3.2 MATERIALS AND METHODS

3.2.1 Study Sites

The study was conducted in Shivaji Nagar primary health centre (PHC) within Samastipur district, Bihar. Four villages (Pura, Bardhiya, Kaji Dumram and Jakhar) were selected based on their high VL case incidence in 2013. A total of 100 houses were visited and sampling was conducted from three randomly selected walls in the bedroom. All wall samples were taken from 2-4ft above the floor.

As part of the VL elimination efforts led by the National Vector Borne Disease Control Programme (NVBDCP), IRS to achieve a target dose of 1g ai/m^2 DDT (wettable powder) was conducted in all four villages. Common surfaces sprayed within this area include lime wash, brick, mud and thatch.

3.2.2 Collection Methods

A total of four collection methods for IRS quality assurance were evaluated, including two pre-spray methods: filter paper (WHO gold standard), felt pads (as reported in the Vanuatu study (Russell et al. 2014), and two post-spray methods: using circular adhesive discs retrieving insecticide either by applying pressure with a sampling tool (Figure 8), or pressing with the ball of the thumb.

3.2.2.1 Pre-spray methods

Whatman Grade 1 filter papers were cut in to 5cm^2 squares, whilst circular shaped felt pads (25mm diameter and 1mm thickness) were obtained from the British Felt Company, Milton Keynes (as previously used in (Russell *et al.*, 2014)).

Filter papers were attached to three walls in the bedroom using the felt pads, as shown in Figure 7, prior to IRS, to ensure they were sprayed together in a single swath and minimise potential for variation. A further four coloured and four white mimic papers were also attached on all four walls at heights ranging from 0-6ft. After IRS, filter papers and felt pads were left to dry for a minimum of two hours, or until dry to the touch, before being collected.

Once dry, all felt pads were peeled off the walls and placed into individual 50ml falcon tubes and stored at 4°C prior to HPLC analysis. Sellotape was used to seal the filter paper coated in insecticide during IRS. Each filter paper was wrapped in aluminium foil, placed into a zip lock bag and stored at 4°C until analysed by HPLC.

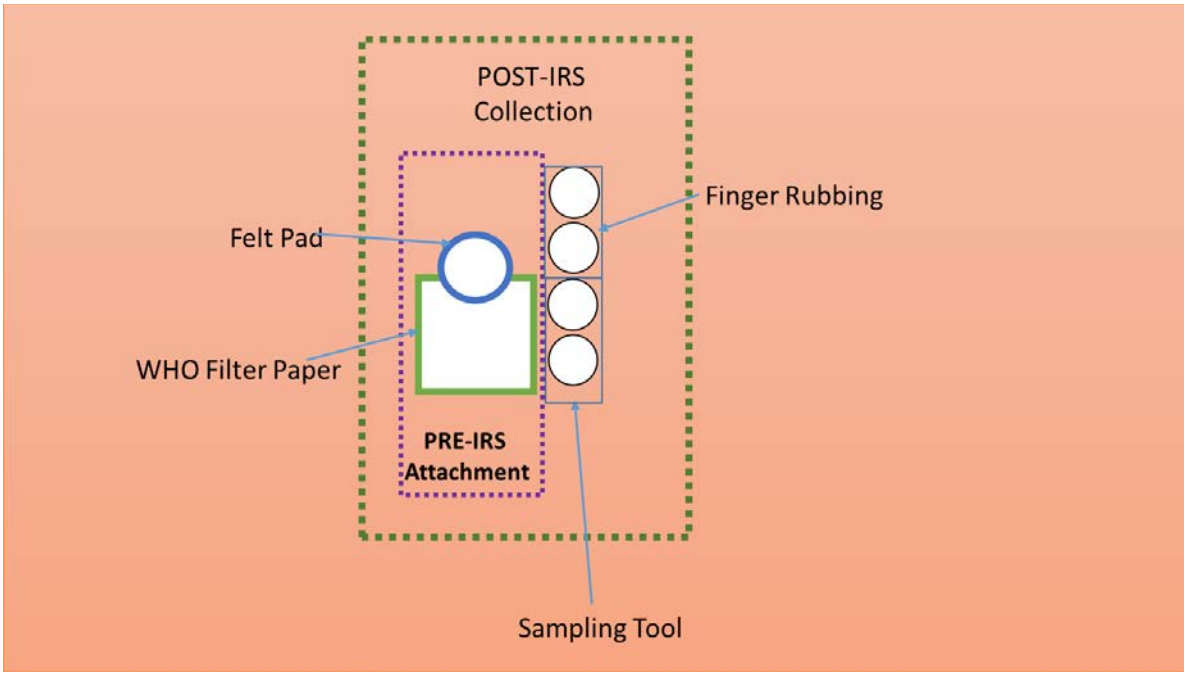

Figure 7: Sample Collection Methods

Image of filter paper, felt pad and adhesive discs are not to scale and are for demonstrating position only.

3.2.2.2 Post-spray methods

Adhesive discs (5cm^2) produced by Bostik were used for the two post-IRS sample collection methods. The discs here used were identified as a potential method for extracting insecticide samples from sprayed walls, as required within the DQK kit prototype (Chapter 4).

Once IRS was complete, and the walls were dry to the touch, two Bostik discs were placed adjacent to the filter paper (as shown in Figure 7) and felt pads and then rubbed on the wall with the ball of the thumb three times (BD Finger rub). The dots were removed using tweezers and placed into 50ml falcon tubes and stored in polythene bags at 4^{o}C for HPLC analysis.

A further two Bostik dots were placed on the wall and then pressed against the surface using the foam sampling tool (BD Sampling tool) until the guard touched the wall as shown in Figure 8. Tweezers were used to remove the Bostik dots and they were stored following the same procedure as used for the BD Finger rub samples. Gloves were worn throughout the sampling process.

Figure 8: Sampling tool for insecticide extraction

3.2.3 Sample analysis

3.2.3.1 Insecticide extraction

3.2.3.1.1 Filter Paper and Bostik dots

Solvent extraction was performed as follows. Filter papers were removed from the aluminium foil, cut into ~1 cm^2 pieces and placed into individual falcon tubes, whilst Bostik dots (both Finger rub and Sampling tool) and felt pads were kept in the falcon tube used during sample collection. One millilitre of a heptane/1-propoanol mixture (9:1) containing 100µg of the internal standard dicyclohexyl phthalate (DCP) was added, after which the tubes were vortex-mixed for 2-3 minutes to extract the *p*,*p'*-DDT. A total of 500µl was aliquoted into clean glass tubes and evaporated to dryness under nitrogen at 40 °C. One millilitre of methanol was added, and the mixture was centrifuged at 22,865 × *g* for 15 min.

NOTE: *para,para*'-DDT (*p,p'*-DDT) is an isomer of DDT. The World Health Organisation specifies that technical DDT intended for use in public health programmes should contain a minimum of 70% *p,p'*-DDT (World Health Organization, 2009).

3.2.3.2 High performance liquid chromatography analysis

HPLC analysis was conducted by injection of 10µl aliquots of DDT extract onto a reverse-phase Hypersil Gold C18 column (75 Å, 250 × 4.6 mm, 5-µm particle size; Thermo Scientific) at 23–25 °C. Acetonitrile/water (93:7) was used for the mobile phase, at a flow rate of 1 mL·min^{-1} to separate DDT and DCP. Standard curves were established for DDT and DCP from known concentrations of authenticated standards. The standard curves were then used to calculate concentrations of unknown samples. Final DDT content in grams per square meter was estimated using the following equation:

A = B × 2 500 × H × D,

where A is p,p'-DDT in grams per square meter, B is p,p'-DDT in micrograms per millilitre obtained from HPLC, H is DDT extraction efficiency by heptane equal to 83.4, and D is the internal standard correction factor calculated from dividing the peak area of 100µg/mL DCP by the DCP peak area obtained for the unknown sample.

3.2.4 Data analysis

All analysis was completed in IBM SPSS Version 26. Comparative analysis of each pre-spray method and post-spray method was done using the Pearson's correlation coefficient.

The sampling efficiency was calculated as a percentage, considering DDT content with post-spray methods versus pre-spray methods using the formula:

$$Sampling\ Efficiency = \frac{Insecticide\ content\ from\ PostSpray\ method}{Insecticide\ content\ from\ PreSpray\ method}\ x\ 100$$

Analysis was done considering the wall surface type and irrespective of surface type.

Pairwise comparisons of insecticide content detected were conducted using a Generalised Estimating Equation (GEE), whereby factor effects in sample type, surface and interaction between surface and sample type were considered.

3.3 RESULTS

A total of 300 matched samples were collected during the study and analysed by HPLC. Samples were taken from all typical Bihar, Indian house wall surface types (brick, mud, thatch, limewash) dependent on availability. The highest number of samples were taken were from brick walls (98), whilst the least number of samples were taken from mud walls (64) (as shown in Table 7).

Table 7: Overview of samples analysed and insecticide extracted by sampling method

	Sample	*Surface*	*#*	*Minimum (g/m2)*	*Maximum (g/m2)*	*Mean (g/m2)*	*s.d.*
Pre-Spray	**Filter Paper**	All surfaces	300	0.02	13.11	0.92	1.03
	Felt Pad	All surfaces	300	0.12	10.96	1.54	1.04
Post-Spray	**BD Finger Rub**	**All surfaces**	**300**	**0.01**	**1.33**	**0.24**	**0.23**
		Thatch	67	0.03	0.57	0.19	0.10
		Limewash	71	0.01	1.33	0.42	0.29
		Mud	64	0.02	1.01	0.30	0.21
		Brick	98	0.01	0.74	0.09	0.12
	BD Sampling Tool	**All surfaces**	**300**	**0.01**	**0.89**	**0.20**	**0.18**
		Thatch	67	0.03	0.57	0.18	0.10
		Limewash	71	0.05	0.89	0.37	0.21
		Mud	64	0.02	0.81	0.25	0.18
		Brick	98	0.01	0.24	0.07	0.05

Target operational dose within the Indian VL elimination programme: 1g/m^2

3.3.1 Pre-Spray Methods

The average concentration of DDT detected with filter papers was 0.92g/m^2, whilst the average dose of insecticide detected using the felt pad was 1.54g/m^2, as shown in Table 7. The standard deviation of DDT on filter paper samples (1.03) was larger than the mean, however this was not the case with felt pads (s.d. = 1.04).

When comparing concentrations of DDT detected between individual felt pads and filter papers, an average of 3.96 (s.d. = 7.38) times higher concentrations of insecticide was detected on felt pads in comparison to filter papers. The smallest difference was 0.12 times higher, and the largest difference was 62.59 times higher.

Using the Pearson correlation coefficient, concentration of insecticide detected using felt pads was shown to correlate to that of the filter papers ($r=0.382$, $df = 298$, $p < 0.001$), however a low coefficient of determination was seen ($r^2 = 0.146$ – Figure 9). An additional Spearman's rank correction was conducted and provided similar results ($r=0.361$, $df = 298$, $p < 0.001$) suggesting the relationship was not driven by outlying values. Due to the nature of sample collection method, whereby insecticide was deposited directly on the filter paper or felt pad, no comparisons between surface types were performed.

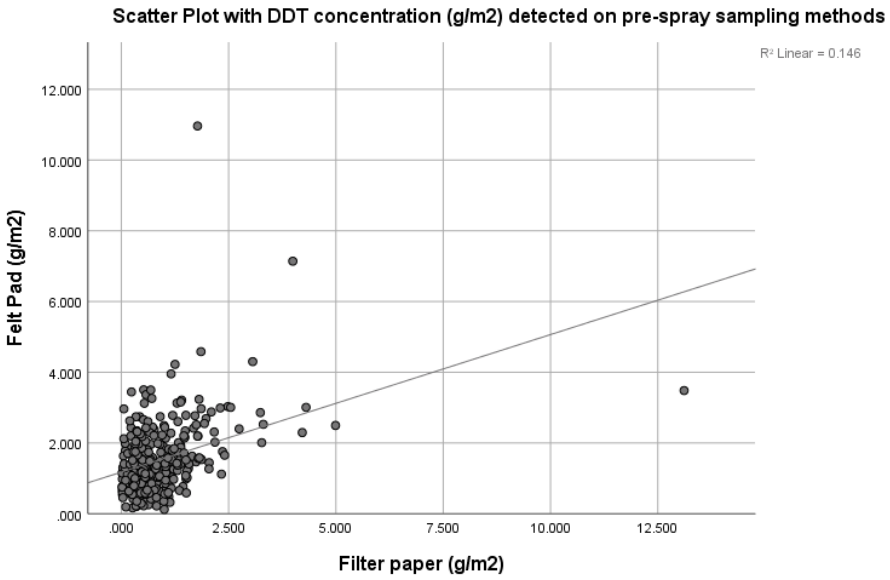

Figure 9: Scatter plot of DDT concentration detected by two pre-spray sampling methods

3.3.2 Post-Spray Methods

The average concentrations of DDT detected using post-spray methods were 0.24 and 0.20g/m^2 for BD Finger rub and BD Sampling tool methods, respectively. The highest concentration recorded post-spray was on samples retrieved using the BD finger rub method (1.33/m^2), however the lowest levels were also detected using the BD Finger rub method and on BD Sampling tool methods (0.01g/m^2).

When including surface type from which post-spray samples were taken, the highest average concentration of insecticide was detected on limewash surfaces irrespective of sampling method, as shown in Table 7. The lowest concentration of insecticide was detected on brick (0.01g/m^2 irrespective of sample collection type) and limewash (BD Finger Rub: 0.01g/m^2).

3.3.2.1 Filter Paper compared to post-spray methods

There was a positive Pearsons correlation coefficient when comparing the filter paper reference method to both post-spray methods (Finger Rub: $r = 0.288$, $df = 298$, $p < 0.001$; Sampling Tool: $r = 0.233$, $df = 298$, $p < 0.001$). As with the pre-spray methods, a Spearman's rank correlation was also performed showing similar results (Finger Rub: $r = 0.496$, $df = 298$, $p < 0.001$; Sampling Tool: $r = 0.463$, $df = 298$, $p < 0.001$). The strength of association for both post-spray methods was weak.

In comparison to the WHO recommended method for sample collection (filter papers), the average sampling efficiency irrespective of surface was 33.37% using the BD Sampling tool method, and 35.59% using the BD Finger rub. The sampling efficiency for both post-spray methods ranged between 0.87- 342.05% for sampling tool and 0.67-409.93% for finger rub. A difference in average sampling efficiency against both pre-spray methods was also seen when the surface from which the sample was retrieved was considered, as shown in Table 8.

3.3.2.2 *Felt Pads compared to post spray methods*

The correlation between felt pads and both post-spray methods was significant (Finger Rub: r = 0.284, df = 298, p < 0.001; Sampling tool: r = 0.183, df = 298, p = 0.001), however the strength of association was also weak (Figure 10 and 11).

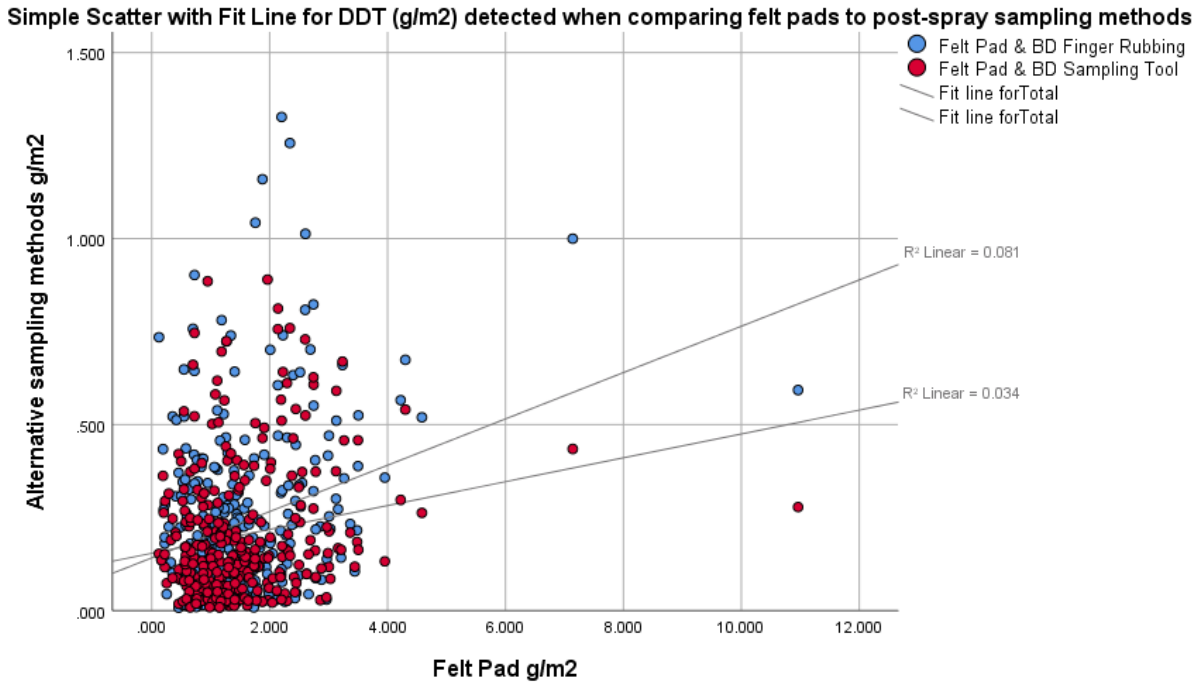

Figure 10: Scatter plot to show association between insecticide detected on Felt pads (pre-spray) versus post-spray sampling methods

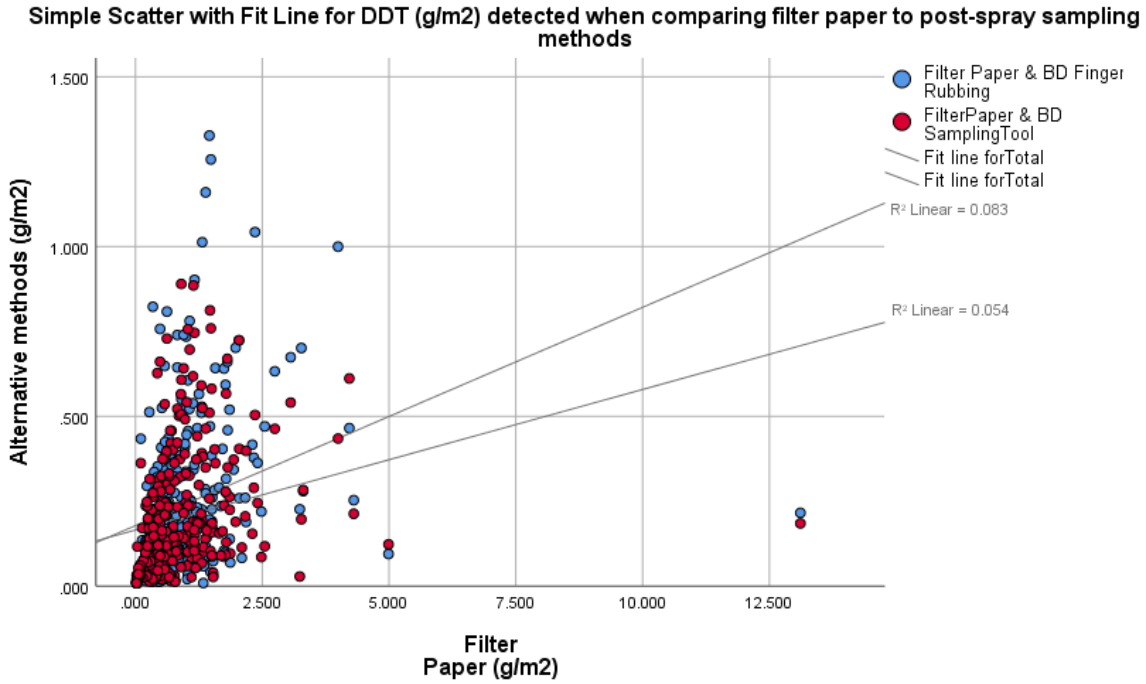

Figure 11: Scatter plot to show association between insecticide detected on Filter paper (pre-spray) versus post-spray sampling methods

When considering the sampling efficiency of the post-spray sampling methods to felt pads, an overall average sampling efficiency of 19.05% was seen for the sampling tool, whilst for the finger rub method an average 23.08% sampling efficiency was seen. The range of sampling efficiency ranged between 0.77% and 189.52% for the sampling tool method, whilst for the finger rub method the range was between 0.52-602.49%.

Table 8: Sampling efficiency of post-spray sampling methods in comparison to pre-spray methods, by surface

Surface	Reference method	Sampling Efficiency (%)									
		BD Sampling Tool					BD Finger Rub				
		No. of sampling points	Mean	Max	Min	s.d.	No. of sampling points	Mean	Max	Min	s.d
ALL	Filter Paper	***300***	***33.37***	***342.05***	***0.87***	***36.28***	***300***	***35.59***	***409.93***	***0.67***	***36.97***
Brick		98	22.95	317.91	0.87	38.81	98	20.01	73.43	1.65	18.01
Limewash		71	42.60	145.76	8.40	29.49	71	47.18	240.25	0.67	37.02
Mud		64	43.23	342.05	4.95	46.60	64	50.77	409.93	7.13	56.23
Thatch		67	29.42	80.97	2.46	19.79	67	31.59	114.95	1.90	22.30
ALL	Felt Pad	***300***	***19.05***	***189.52***	***0.77***	***24.39***	***300***	***23.08***	***602.49***	***0.52***	***43.38***
Brick		98	7.66	125.28	0.77	15.30	98	13.52	602.49	0.80	61.22
Limewash		71	28.18	130.97	2.54	25.12	71	31.02	134.97	0.52	28.26
Mud		64	28.11	189.52	1.62	30.15	64	34.01	227.14	1.40	37.76
Thatch		67	17.36	130.86	1.35	21.20	67	18.24	126.67	1.90	21.60

3.3.3 Generalised estimating equation

Multiple post-hoc pairwise comparisons were conducted to compare between and within group means (Table 9). The comparison of pre-spray methods (filter paper and felt pad) showed a significant difference ($p<0.001$).

As shown in Table 9, the concentration of insecticide detected using both pre-spray methods (filter paper and felt pad), irrespective of surface type, were significantly different to both post-spray methods ($p < 0.001$). Surface type was also included to compare pre-spray methods to post-spray methods. A statistically significant difference was seen for all surfaces and sample collection method comparisons (e. g. $p < 0.001$ for both post-spray sampling methods (Bostik Finger Rub and Bostik Sampling Tool) on brick surfaces in comparison to filter paper).

Table 9: GEE approach analysis result for pre-spray and post-spray sampling methods

Surface	Sample type (I)	Sample Type (J)	Mean Difference (I-J)	Std. Error	df	p-value*	95% Wald Confidence Interval for Difference	
							Lower	Upper
Overall	felt pad	Bostik Finger Rub	1.283[a]	0.079	1	0.000	1.074	1.491
		Bostik Sampling Tool	1.317[a]	0.081	1	0.000	1.104	1.531
		filter paper	0.609[a]	0.070	1	0.000	0.424	0.793
	filter paper	Bostik Finger Rub	0.674[a]	0.067	1	0.000	0.496	0.851
		Bostik Sampling Tool	0.708[a]	0.070	1	0.000	0.524	0.893
		felt pad	-0.609[a]	0.070	1	0.000	- 0.793	- 0.424
Brick	felt pad	Bostik Finger Rub	1.378[a]	0.112	1	0.000	0.981	1.775
		Bostik Sampling Tool	1.404[a]	0.116	1	0.000	0.995	1.814
	filter paper	Bostik Finger Rub	0.733[a]	0.167	1	0.001	0.145	1.322
		Bostik Sampling Tool	0.760[a]	0.173	1	0.001	0.150	1.369
Limewash	felt pad	Bostik Finger Rub	1.506[a]	0.236	1	0.000	0.673	2.339
		Bostik Sampling Tool	1.561[a]	0.243	1	0.000	0.702	2.421
	filter paper	Bostik Finger Rub	0.713[a]	0.117	1	0.000	0.300	1.127
		Bostik Sampling Tool	0.769[a]	0.125	1	0.000	0.326	1.212
Mud	felt pad	Bostik Finger Rub	0.977[a]	0.133	1	0.000	0.507	1.447
		Bostik Sampling Tool	1.027[a]	0.137	1	0.000	0.544	1.510
	filter paper	Bostik Finger Rub	0.498[a]	0.087	1	0.000	0.190	0.807
		Bostik Sampling Tool	0.549[a]	0.098	1	0.000	0.204	0.893
Thatch	felt pad	Bostik Finger Rub	1.157[a]	0.128	1	0.000	0.707	1.608
		Bostik Sampling Tool	1.207[a]	0.127	1	0.000	0.759	1.656
	filter paper	Bostik Finger Rub	0.750[a]	0.148	1	0.000	0.226	1.273
		Bostik Sampling Tool	0.757[a]	0.149	1	0.000	0.232	1.281

3.4 DISCUSSION

The WHO recommended method for collecting insecticide samples for IRS quality assurance is problematic due to operational issues in securing the filter papers to the wall, and spray operators becoming sensitised to the performance assessment method (Russell *et al.*, 2014). Furthermore, the use of filter papers only allows the amount of residual insecticide to be assessed at the time of spraying.

Three alternative methods of insecticide sample collection were evaluated against the WHO recommended method. Whilst the felt pad discs were considered less conspicuous than white filter papers and could easily be missed by spray operators, the post-spray bostik discs allowed sample collection to be unaffected by behavioural bias as the location from where the sample would be taken would not be decided until after the wall was sprayed.

Spray operator performance was variable as assessed by both pre-spray sample collection methods (Table 7). Filter papers had a range of 0.02g/m^2 - 13.11g/m^2 compared to the target dose of 1gm/m^2. The range for the felt pads was 0.12g/m^2 - 10.96/m^2. The ranges between methods were very similar, and most likely due to the study design; where felt pads and filter papers were placed in close proximity to each other, therefore if spray personnel targeted the filter paper during spraying, the felt pads would also be sprayed with the same level of insecticide.

Pearsons' correlation coefficient demonstrated that there was a significant association between filter papers and felt pads, however the strength of association was low (r^2= 0.146). A GEE approach was used to test for an association between insecticide concentration and detection method, which found that insecticide content detected on filter papers were significantly different in value to felt pads (GM ratio: -0.496, df = 1, p< 0.001) and therefore could not form an effective surrogate to the gold standard method. That said, the felt pad is smaller and less conspicuous, and therefore could be taken forward independently to determine if it is a better predictor of variable spray quality, with no association to the filter paper readings.

The use of filter papers for assessing insecticide deposits is widely accepted as the standard within WHO guidelines and considered operationally feasible, from IRS quality assessment to field trials for new operational formulations (World Health Organization, 2006a, 2019). Whilst the method is not affected by the surface type, the filter papers were prone to moving during IRS and coming into contact with the wall surface. Where poor IRS practices were followed, this may lead to insecticide running down the wall and being absorbed onto affixed filter papers, presenting an inaccurate representation of the IRS performance. The felt pads were smaller and more inconspicuous, as home owners typically had posters, religious images and calendars attached to the wall and a white circular dot could be considered a remnant of those items. However, the sample collection method is still prone to similar sample collection issues, and due to the thickness of the felt pad and material properties, was found be more highly absorbent. Results from the pairwise comparisons suggest insecticide deposition during IRS is highly variable, and the two pre-IRS collection methods placed side by side still provided highly variable data.

Post-spray methods were compared against both the filter paper and felt pad methods. The Pearsons' correlation co-efficients showed a significant positive correlation between both pre-spray methods and post-spray methods. However, a low strength of association was also observed. The GEE approach showed insecticide recovered from filter papers were significantly different to that recovered in both post spray methods (Bostik Finger Rub: GM ratio: -0.395, df = 1, $p < 0.001$; Bostik Sampling Tool: GM ratio: -0.345, df = 1, $p < 0.001$). Similarly significant differences in insecticide detected from felt pads in comparison to the two post-spray methods were observed (Bostik Finger Rub: GM ratio: 0.249, df = 1, $p < 0.001$; Bostik Sampling Tool: GM ratio: 0.275, df = 1, $p < 0.001$). The significant differences were also observed when the data was disaggregated by surface area, indicating that previous reports of extraction efficiency from different wall surfaces remained an issue with both Bostik dot collection methods (Russell *et al.*, 2014).

Sampling efficiency for post-spray methods with the Bostik dot were highly variable when compared to both filter papers and felt pads, further confirming results shown in the GEE model and suggesting that a correction factor could not be applied. As reported previously, the sampling efficiency of post-spray methods have been highly variable using other extraction methods, such as Sellotape.

The study aimed to identify an alternative sampling method to Whatman Grade 1 filter papers for IRS QA. Felt pads are a possible alternative, as they are less conspicuous, and may be an alternative to remove potential behavioural bias. However, both methods generated variable results and are still reliant on HPLC analysis which is expensive (estimated at ~£3/assay) for operational use for most IRS programmes (Ismail *et al.*, 2016). In addition, there is a requirement for specialist trained staff and regular servicing of sensitive HPLC equipment, which may not be feasible in resource-poor settings. Post-IRS sample collection issues associated with sampling efficiency and surface type have been reported extensively, therefore pursuing alternative IRS QA methods which allow the insecticide to be measured directly on the wall with the need for prior extraction would be advantageous. The limitation for pre-spray methods will remain that data sets can be collected from only one timepoint and residual insecticide cannot be measured over time.

4 Field Validation of the Dichlorodiphenyltrichloroethane (DDT) Insecticide Quantification Kit in Bihar, India

Research conducted in Chapter 3 and 4 are linked, as both are assessing alternative methods for assessing the insecticide dose deposited on to walls during IRS. The post-spray adhesive discs were used in the DDT quality assurance kit prototype, however the two chapters demonstrate issues with using this method notably the issues with sampling efficiency and therefore poor reliability.

Contributions:
Rinki was responsible for developing the study design, developing study protocols and for providing training to the team members on following the manufacturer's instructions for use. Whilst field teams collected the samples, Rinki was responsible for supervising and leading the field teams, providing retraining, and for ensuring the protocols were adhered to throughout the field survey. Analysis of data produced from using the prototype and interpretations of results were done by Rinki Deb, with statistical guidance from LSTM statistician, Agnes Matope. Dr Mark Paine provided supervisory guidance on research design and results interpretation. Field teams involved in the sample collection were from Rajendra Memorial Research Institute, including Dr Rudra Pratap Singh who supported field implementation of the survey. Dr Pushkar Shivam supported with some HPLC analysis of samples collected from the field.

4.1 INTRODUCTION

4.1.1 Quality Assurance of Indoor Residual Spraying

Vector control is a cornerstone intervention in interrupting transmission of vector borne diseases such as malaria and visceral leishmaniasis. The success of interventions such as IRS and long-lasting insecticidal nets (LLINS) is dependent on the vector being exposed to the optimal dose of insecticide so that control of the vector population can be achieved. Effective monitoring strategies and implementing strong quality control procedures within the programme is essential in achieving this (World Health Organization, 2015; Ismail *et al.*, 2016).

The insecticide quantification method accepted for IRS is HPLC from pre-affixed filter papers, wall swabs or by removing sticky-tape samples (Brown and Hartwick, 1988; Russell *et al.*, 2014). Data generated from this assessment should be coupled with cone bioassays to assess the residual efficacy of the insecticide available on the surface to the vector (Silver, 2008).

Insecticide quantification methods are operationally challenging due to the requirement of highly skilled staff and specialist equipment. Analysis by HPLC is expensive and due to the long data turnaround times is typically not usable for immediate feedback and improvement of performance within current spray activities (Russell *et al.*, 2014). While filter papers are an effective method of collecting samples independent of wall surfaces, they are visible to spray operators and repeated surveys can lead to bias and results being unrepresentative of actual doses delivered on walls. The

filter paper method also prevents any repeated analysis of insecticide delivered, which would inform the programme of insecticidal decay rates, which is also a requirement for monitoring spray quality (Russell *et al.*, 2014).

When considering sampling by wall scrapings, swabs or removal of sticky tapes: the methods have been reported to suffer from issues associated with variability of the wall surface, difficulties in obtaining samples from surfaces and extraction efficiencies that could not be controlled (Morou *et al.*, 2008). Studies in Vanuatu reported Sellotape to lift low quantities of insecticide and low extraction efficiency (~10% of applied insecticide). However, this was more tractable for collecting samples of poorly absorbent wettable powder formulations, such as for DDT (Barlow, 1955; Dowd *et al.*, 2009).

Residual efficacy assessment by cone bioassay also presents with operational issues, namely the requirement of an established fully susceptible insect colony and an insectary able to provide large numbers of the vector annually to support testing efforts. In the absence of skilled staff, the results may also be unreliable due to inexperience and human error while performing the test.

Colorimetric assays, developed as an alternative method to support quality assurance, have been implemented in countries including Vanuatu, Tanzania, Ethiopia and Bioko Island for a range of different insecticide classes and demonstrated promising results (Russell *et al.*, 2014; Protopopoff *et al.*, 2015; Yewhalaw *et al.*, 2017; Fuseini *et al.*, 2020). Testing using these assays could be done *in situ*, providing the opportunity for rapid feedback and improvement of the spray-operator performance. Despite success implementing the tools within a research context, there has been limited support for widescale adoption of the technologies by the World Health Organisation.

4.1.2 Indoor residual spraying and quality assurance in India

The Directorate of National Vector-Borne Disease Control Programme (NVBDCP) within the Ministry of Health and Family Welfare is responsible for implementation of IRS in India. The 2016 Integrated Vector Management (IVM) strategy outlines IRS as an effective control method for malaria and visceral leishmaniasis (VL), with the first insecticide of choice being 50% dichlorodiphenyltrichloroethane (DDT) sprayed at 1g/m^2 dosage (Directorate National Vector Borne Disease Control Programme, 2016b).

More specifically, for malaria control, the strategy should be implemented only in human dwellings, and where DDT resistance has been reported, malathion and synthetic pyrethroids are listed as alternative options. For VL, the strategy targets both human dwellings and animal shelters, spraying the walls to a height of six feet. Similar to malaria, in areas of DDT resistance, pyrethroids are the alternative insecticide class of choice (Directorate National Vector Borne Disease Control Programme, 2016b).

Within the monitoring and evaluation section of India's IVM strategy document, entomological indicators are clearly outlined for effective disease impact monitoring. However, no programme process or performance indicators, aside from coverage, were specified for IRS. Therefore, quality assurance of insecticide during IRS operations was not defined as a programme requirement (Directorate National Vector Borne Disease Control Programme, 2016b). This is reflective of typical practices within vector control programmes globally, especially where programmes are nationally funded and the focus is on delivery of insecticidal products in targeted structures, with very limited resources available to monitor the quality of delivery.

A study conducted in 2011 by Chowdhury *et al.* in India and Nepal analysed filter papers affixed prior to IRS, by standard quantitative gas chromatography; individual filter paper results from India showed extreme variation in the dose delivered (9.1-330% of the target concentration), supporting the need for close monitoring of the quality of IRS and effective tools to enable rapid assessment (Chowdhury *et al.*, 2011), but this did not result in any changes in the National Programme.

4.1.3 Insecticide quantification kit for DDT

The Liverpool School of Tropical Medicine in partnership with IVCC developed a suite of field friendly, inexpensive and simple methods to quantify insecticide concentrations for a range of insecticide classes used in bed nets and sprayed surfaces (IVCC, 2013; Liverpool School of Tropical Medicine, no date). These tools were developed with the operational environment in mind; meeting the demand for rapid assessment and an effective feedback loop system to enable rapid response to suboptimal spray procedures. Field trials in Vanuatu with the cyanopyrethroid quantification kit demonstrated the feasibility of such processes (Russell *et al.*, 2014).

As part of the suite of tools, a colorimetric DDT quantification kit (DQK) was designed for supporting quality assurance of IRS in areas where this insecticide was used for vector control (Ismail *et al.*, 2016). Until 2015, DDT was the insecticide of choice for VL vector control in India, providing the opportune environment for field testing and validation of the kit.

4.1.4 Study aims

The aim of this chapter is to determine if the final DQK prototype could be implemented operationally to support IRS quality assurance and provide comparable results to HPLC analysis.

4.2 MATERIALS AND METHODS

4.2.1 Study sites

The study was conducted across three primary health centres (PHCs) within Samastipur district, Bihar: Kalyanpur, Bithan and Mohanpur. Two VL endemic villages per PHC, scheduled to receive IRS as part of the National Vector Borne Disease Control Programme (NVBDCP), were randomly selected. A total

of 45 houses, across the six villages, were recruited as part of the study, ensuring all typical surfaces were included (brick, mud, thatch and limewash). All wall samples were taken from 2-4ft above the floor.

4.2.2 Sample collection and analysis

Within each house, three adjacent walls were selected, whereby the first wall was randomly selected using the spin the bottle method.

4.2.2.1 DDT insecticide quantification kit

After IRS, the sprayed surfaces were left to dry for a minimum of two hours. Once dry, insecticide residue samples were taken following the user instructions provided within the DQK prototype (Annex 1). Two adhesive circles were placed on to the sprayed wall side by side, as shown in Figure 12. The sampling tool was placed over the first adhesive circle and pressed down until the guard made contact with the wall. The sampling tool was removed from the wall and reapplied on to the second disc, following the same methodology. Using tweezers, the two discs were removed and placed into a sample jar, labelled to note where the sample had been retrieved from.

Collected samples were then transported back to the field laboratory and analysed as per the instruction leaflet (Annex 1) using reagents provided within the prototype kit. Briefly, 1ml of Reagent A (heptane) was added to the sample bottle, ensuring both adhesive discs were covered in the solution. The jar lid was replaced, and the bottle shaken for 30 seconds. The sample bottle was then left to stand for two minutes, with intermittent shaking to maximise insecticide extraction. After two minutes, 0.68ml of the liquid (heptane containing DDT), was extracted using the syringe and transferred into a glass bottle. 0.1ml of Reagent B (2M potassium hydroxide dissolved in 1-propanol) was added to the glass bottle and once the lid was replaced, the bottle was mixed well. The glass receptacle was then left to stand for one hour, with intermittent shaking (approximately every 15 minutes). After an hour had passed, the lid was removed and 0.1ml of Reagent C (2M acetic acid) was added, the lid replaced and the bottle mixed vigorously. The bottle was then left to stand for a further one minute, after which the Quantab strip was placed into the bottle, ensuring the wick was at the bottom of the aqueous phase. Once the dipstick indicated that it had reached point of saturation, the value on the strip was read and the results converted using the DDT quantitative scale.

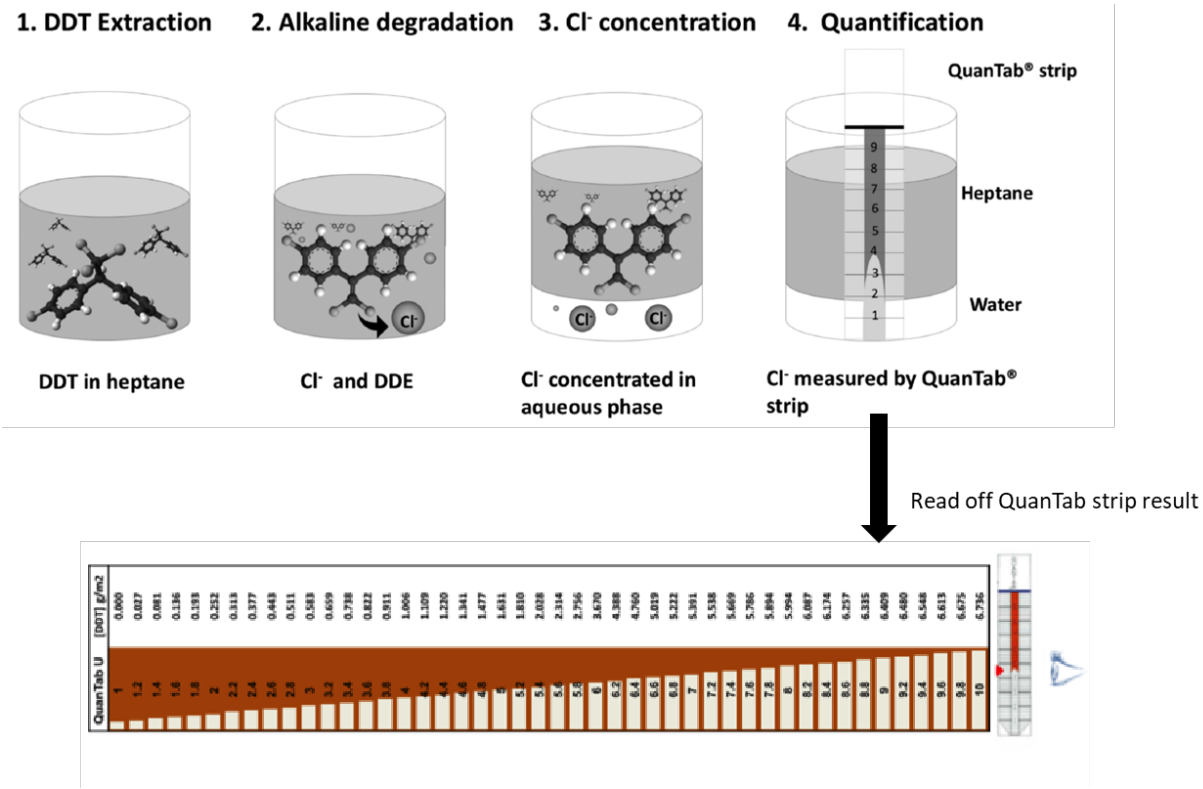

Figure 12: Schematic graph of the dipstick assay for determination of DDT (Ismail et al.*, 2016)*

Results were firstly entered into a results sheet provided within the DQK prototype box and secondly into excel for further analysis.

4.2.2.2 Bostik dots and high-performance liquid chromatography

Bostik dots, identical to those provided in the DQK prototype, were placed adjacent to the DQK dots to retrieve residual insecticide samples from the walls (as shown in Figure 12). The user instructions (Annex 1) provided within the prototype were followed until Bostik dot retrieval, after which dots were placed in 50ml falcon tubes for HPLC analysis. High performance liquid chromatography was performed on the Bostik dots following the procedure outlined in Section 3.2.3.2.

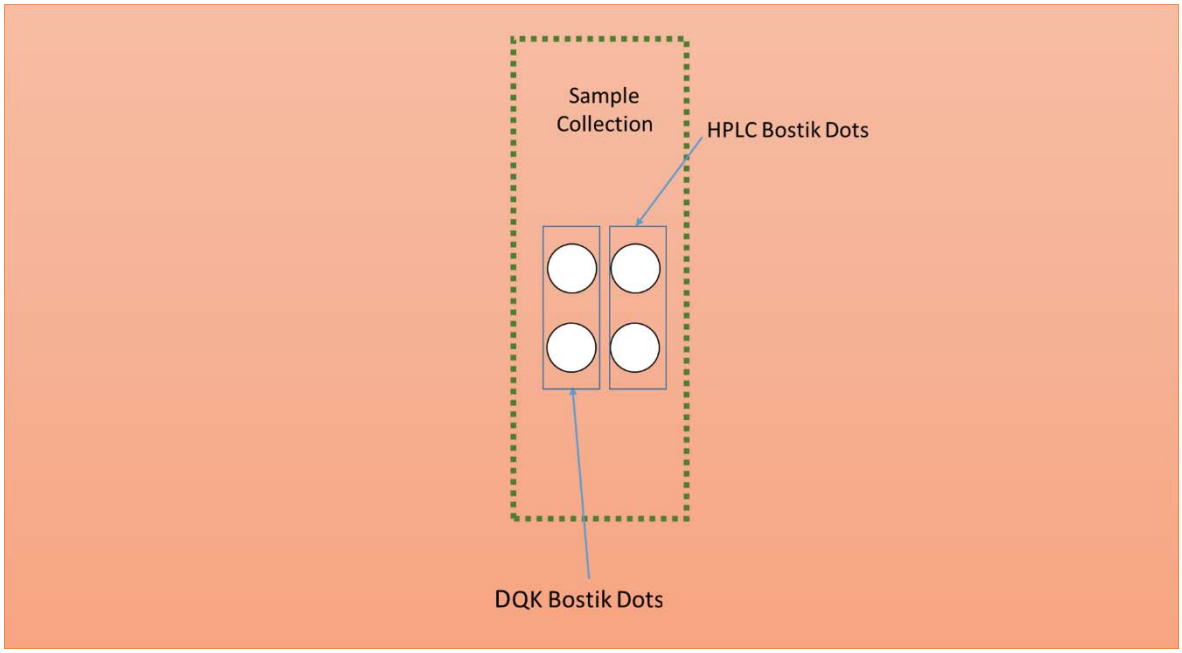

Figure 13: Position of Bostik dots taken for analysis using DQK Prototype and HPLC methods

4.2.3 Data analysis

All analysis was completed in IBM SPSS Version 26. The generalised estimating equation (GEE) approach with an exchangeable correlation matrix and linear method was used to compare the differences between IRS QA sample testing methods. This was followed by the Bonferroni method for pairwise comparisons. Factor effects considering the interaction between surface and sample type was considered. The statistical significance threshold was set as p=0.050, and 95% confidence intervals were used.

4.3 RESULTS

A total of 128 matched samples were taken from a total of six villages and from the four typical surface types: brick (39), limewash (39), mud (30) and thatch (20). To remove the potential for user bias, a total of six technicians were trained on how to use the DQK prototype and were responsible for collecting both HPLC and DQK samples and performing the DQK test from sample collection to test result interpretation. A total of nine prototype boxes were used and all DQK tests were conducted in a field laboratory on the day of sample collection, and all DQK testing was complete in two days.

4.3.1 DQK versus HPLC test analysis

The average quantity of insecticide detected with the two methods, DQK and HPLC, can be seen in Table 10: the average detected content of insecticide by the DQK prototype was higher than that observed in the WHO gold standard method across all surface types. No DQK result was available for nine of the samples analysed, in addition 0g/m^2 insecticide was detected using the DQK prototype from 22 samples analysed, where the corresponding HPLC result did detect insecticide. Some

Insecticide concentrations ranged from 0.01-6.35g/m^2 using the HPLC method across all samples (Fig 13).

Table 10: Average insecticide detected by method (HPLC or DQK) and by surface type

Surface Type	DQK					HPLC				
	Average insecticide quantity detected (g/m2)	s.d	95.0% Lower CL for Mean	95.0% Upper CL for Mean	Standard Error of Mean	Average insecticide quantity detected (g/m2)	s.d	95.0% Lower CL for Mean	95.0% Upper CL for Mean	Standard Error of Mean
Brick	0.576	0.752	0.322	0.831	0.125	0.206	0.157	0.153	0.259	0.026
Limewash	0.958	0.992	0.622	1.294	0.165	0.793	1.084	0.426	1.159	0.181
Mud	1.744	1.148	1.299	2.189	0.217	0.870	0.691	0.602	1.138	0.131
Thatch	0.658	0.388	0.472	0.845	0.089	0.516	0.516	0.268	0.765	0.118
All surfaces	**0.980**	0.994	0.799	1.160	0.091	**0.589**	0.763	0.451	0.728	0.070

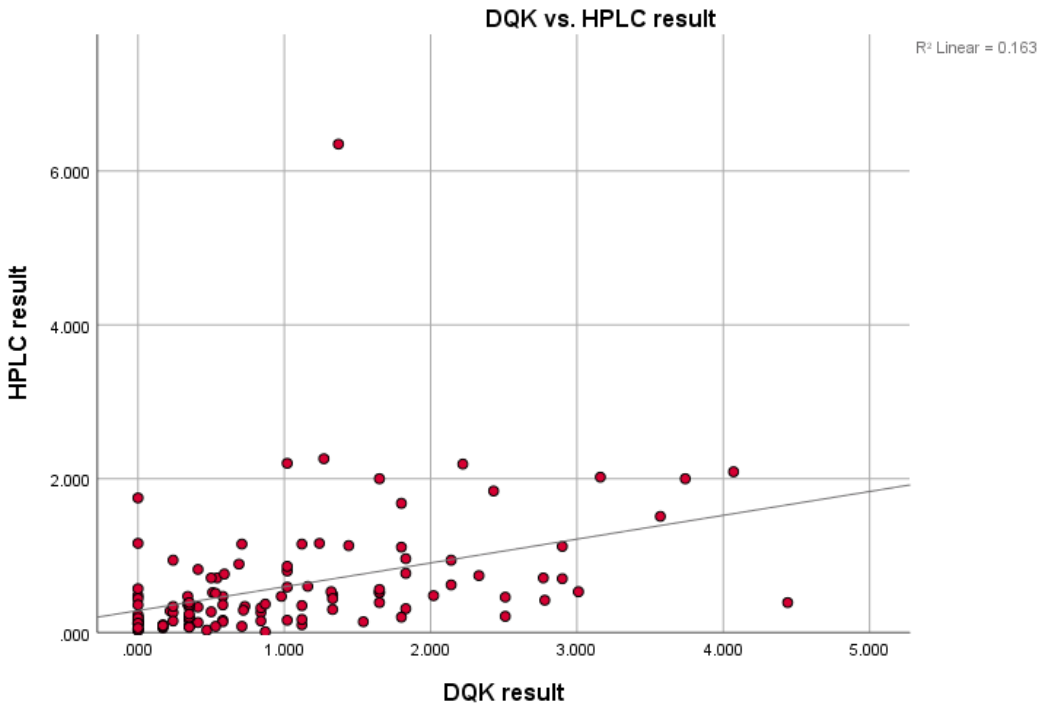

Figure 14: By sampling point, comparison of DQK vs. HPLC result

As shown in Figure 13, one outlier was identified which was removed from the dataset. This showed a mild impact on the trends seen in the correlation, as shown in Figure 14.

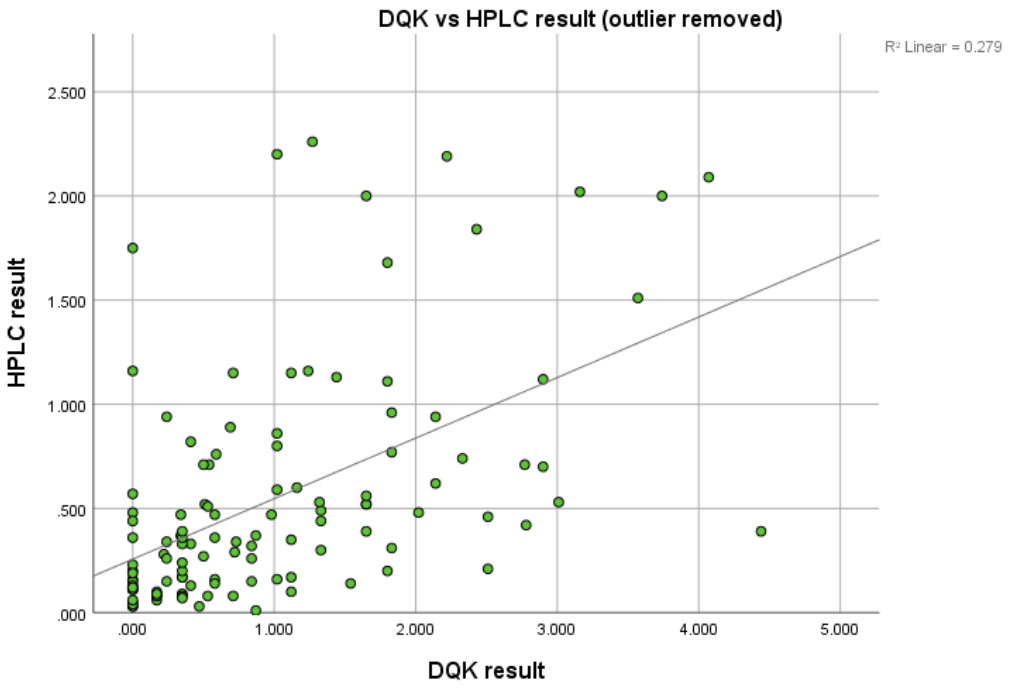

Figure 15: By sampling point, comparison of DQK vs. HPLC result with one anomalous result removed

After removing the samples where no DQK result was available, a Pearson correlation coefficient when comparing DQK to the HPLC method showed a statistically significant difference in results ($r = 0.528$, $df = 116$, $p < 0.001$). This was also seen in the Spearman's rank ($r = 0.576$, $df = 116$, $p < 0.001$) suggesting the relationship was not driven by outlying values. The strength of association for both post-spray methods was weak.

Pairwise comparisons were conducted using the GEE approach (Table 11). A significant difference was detected between HPLC and DQK for samples retrieved from brick (p=0.003, CI: 0.072-0.669) and mud surfaces (p<0.001, CI: 0.508-1.241). No statistical difference was detected on limewash (p=1.000, CI:-0.494-0.825) and thatch (p=1.000, CI:-0.096-0.381) surfaces. When considering the two test methods, but not including the surface interaction, a significant difference between DQK and HPLC was detected (p<0.001, CI: 0.255-0.521).

Table 11: GEE approach result when comparing DQK with HPLC result

Interaction		Mean Difference (I-J)	Std. Error	df	p-value	95% Wald Confidence Interval for Difference		Significant at the .05 level
Surface and DQK result (I)	Surface and HPLC result (J)					Lower	Upper	
Brick*DQK	Brick*HPLC	0.370	0.096	1	0.003	0.072	0.669	*
Limewash*DQK	Limewash*HPLC	0.165	0.211	1	1.000	- 0.494	0.825	
Mud*DQK	Mud*HPLC	0.875	0.117	1	0.000	0.508	1.241	*
Thatch*DQK	Thatch*HPLC	0.142	0.076	1	1.000	- 0.096	0.381	

4.3.2 Prototype observations

Several performance issues associated with the kit prototype were encountered when conducting the DQK tests. To ensure the aim of the study was fully addressed, issues faced when performing the testing were recorded. Issues observed and outlined affected all insecticide quantification tests conducted using the DQK prototype.

Sample collection pot failure

Sample collection pots included within the DQK prototype cracked and became opaque after the addition of the first kit reagent (as shown in Figure 15). A loss of reagent and insecticide was encountered during field laboratory testing, which caused issues in completing the DQK test – primarily reduced or no solution (Reagent A) , so that insecticide extracted from the kit dots, could not be taken forward to latter phases of the DQK protocol. This failure was observed in all DQK kit sample collection pots used within the study.

Figure 16: Cracked and opaque sample collection pots

Wear and tear of sampling tool.

The sampling tool included within the test kit lost some of their properties after repeated use, namely the foam did not expand back to above the guard, preventing appropriate pressure to be applied on to the Bostik dots once attached to the wall. Additional sampling tools were provided, each used to press a maximum of 10 Bostik dots before being replaced.

Kit provided syringe failure

Single syringes were provided within the protype for conducting all testing within a single kit, whereby a total of 20 tests could be performed. After repeated extraction and release of reagents into the sample collection pots, the plunger detached from the seal (Figure 16). In order for testing to continue, additional syringes were procured. This issue affected all syringes (three per kit) in all nine kits used within the field survey.

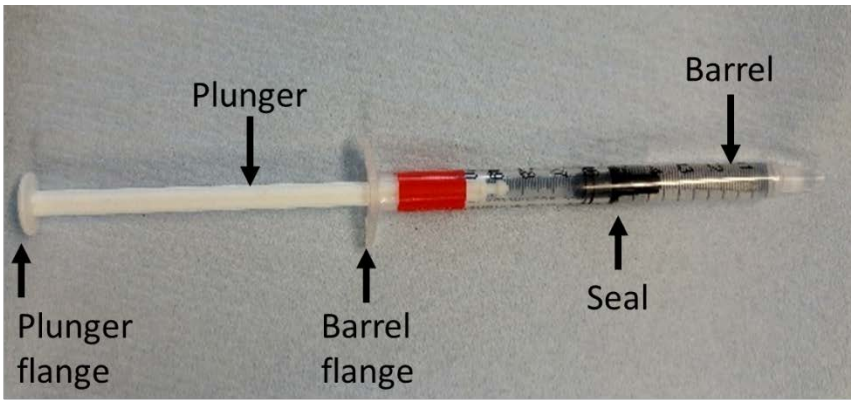

Figure 17: Syringe failure within the DQK prototype

Degradation of reagent pot seal

In two DQK prototype kits the seal for Reagent B was degraded and the colour of the solution within the bottle was not clear, as seen in other kits (Figure 17). The cause and potential impact on kit performance was unknown. All kits were stored at room temperature, as per kit instructions and were used within 2 months of manufacture.

Figure 18: Images of reagent lids degraded and solution colour change

DISCUSSION

Indoor residual spray quality assurance is operationally problematic as the current WHO recommended method for analysis requires expensive equipment and highly trained personnel to perform HPLC (Russell *et al.*, 2014). As a result, quality assurance using this method is often considered not feasible and performance monitoring for IRS is primarily considering coverage rates. However, it is essential to understand if the target dose of insecticide has been delivered and critically

assess effectiveness of the vector control strategy. As reported in Coleman et al. (2015), suboptimal delivery of DDT during IRS could be one of the key factors to explain operationally relevant insecticide resistance in Indian disease vectors such as *Phlebotomus argentipes* sand flies (Coleman *et al.*, 2015).

The DQK provides an operationally friendly alternative way to quantitatively determine the dose of insecticide deposited on to surfaces during IRS. The kit does not require any additional equipment or reagents, outside of what is provided in the box, and results available within an hour of starting the test. Provided that the DQK test results are comparable to other recommended methods of analysis, the testing method could be adopted and become more operationally feasible for countries conducting IRS to control vector species and allow for rapid feedback to IRS teams during the spray round.

However, the multiple issues with the prototype kit plasticware and consumables mean that the prototype in its current format is unsuitable for operational introduction in this format. Further development of this DDT specific kit may not be feasible, due to the phasing out of DDT for IRS globally, meaning the market for such a kit is now very limited. In its current format the field assessment showed a significant difference in residual insecticide detected through filter papers and HPLC versus DQK. It should however be noted that the two analysis methods did not have a statistically significant difference when considering thatch and limewash surfaces. In order for the DQK to be adopted as an alternative method for IRS quality assurance, results obtained from the kit, irrespective of surface type, would have needed to be comparable to HPLC analysis.

Overall, higher concentrations of insecticide were detected through the DQK versus the WHO recommended method. The difference in insecticide content detected could be attributed to the failure of the test kit components, whereby the cracking of the sample collection pot after addition of Reagent A could have been the major point of failure. Lower volumes of Reagent A with extracted insecticide to take forward to the rest of the test may have affected the insecticide content available for the rest of the test. However, it is unclear at which point the cracking occurred and whether this was upon initial addition of reagent A, at which point the insecticide would not have been extracted from the Bostik dot, or later.

The failure of the sampling tool may have allowed for differences in insecticide retrieved from the wall despite samples being taken adjacent to one another. However, insecticide delivery during IRS can be variable, even when comparing samples taken from the same swath (as noted in Chapter 2). Whilst the failure of the syringe would have been unlikely to impact on test performance, from a usability perspective, an alternative method to remove known volumes of solution from the reagent's bottles would need to be considered.

When collecting samples from the walls, a variety in condition of each surface type was noted – for example, some mud walls were more recently replastered with mud, whilst others were flakier and drier. Similarly, limewash walls, dependent on housing quality and location could be affected by moisture and as a result peeling off easily, or in rooms with good ventilation and dry to the touch. The condition of the wall and surface type could affect the level of insecticide that could be retrieved using the Bostik dot collection method, which coupled with the variability of spray within a single swath, could mean a post-IRS sample collection method will be problematic.

The benefit of a post-IRS collection method is to avoid spray operator behaviour bias, which is an inherent issue when continually using pre-spray filter papers attached to walls (Russell *et al.*, 2014). However, the benefit of using filter papers, is that the surface upon which attached, cannot influence the amount of insecticide sprayed onto the paper. For the DQK prototype assay usability to be further assessed, it would be advantageous to determine the kit performance when residual insecticide deposited on to filter papers are assessed using the kit versus HPLC, removing the issues associated with insecticide retrieval using the Bostik dot. If used to assess post-spray, prototype issues would need to be resolved with replacement of components namely the sample collection vessel and the syringe. When considering that quality assurance practices to quantitatively assess spray performance is often ignored within a programme setting, the use of rapid diagnostic kits with filter papers could assist with appropriate performance assessment and encourage continual assessment and improvement, without the need for expensive laboratories, specialist equipment and highly trained staff.

However, the multiple issues with the prototype kit plasticware and consumables mean that the prototype in its current format is unsuitable for operational introduction in this format. Further development of this DDT specific kit may not be feasible, due to the phasing out of DDT for IRS globally, meaning the market for such a kit is now very limited.

5 Monitoring impact of indoor residual spraying to eliminate visceral leishmaniasis in India

This chapter has been published in PLOS Neglected Tropical Diseases, reference below:

Deb R, Singh RP, Mishra PK, Hitchins L, Reid E, Barwa AM, Patra D, Das C, Sukla I, Srivastava AK, Raj S, Mishra S, Swain M, Mondal S, Mandal U, Foster GM, Trett A, Garrod G, McKenzie L, Ali A, Morchan K, Chaudhuri I, Roy N, Gill NK, Singh C, Agarwal N, Sharma S, Stanton MC, Hemingway J, Srikantiah S, Coleman M. Impact of IRS: Four-years of entomological surveillance of the Indian Visceral Leishmaniases elimination programme. PLoS Negl Trop Dis. 2021 Aug 9;15(8):e0009101. doi: 10.1371/journal.pntd.0009101. PMID: 34370731; PMCID: PMC8376195.

Over this time period, the National Programme had switched from DDT to alpha-cypermethrin for IRS as a direct result of the data generated by the Bill and Melinda Gates funded LSTM technical assistance programme to the NVBDCP VL elimination programme.

Contributions:

Rinki was responsible for developing the study design, developing study protocols and for providing training to the team members. Whilst field teams collected the samples, Rinki was responsible for supervising at the start of the study and conducted regular spot checks throughout the study period. Rinki was also responsible for developing quality control systems for onward data quality checking, providing retraining. Any changes to the protocol e.g. change in house or village, were organised through a work flow developed by Rinki and she also led random selection of new villages. Analysis of data produced from using the prototype and interpretations of results were done by Rinki Deb, with statistical guidance from LSTM statistician, Dr Michelle Stanton. Entomological surveillance officers (Miss Arti Barwa, Mr Debanjan Patra, Miss Chandrima Das, Mr Indranil Sukla, Mr Ashish Kumar Srivastava, Miss Shilpa Raj, Miss Swikruti Mishra, Miss Madhuri Swain, Miss Swapna Mondal and Miss Udita Mandal) employed through CARE India were responsible for supervising day to day field site activities and field based sample processing. Laboratory based analysis was conducted by Miss Lisa Hitchins, Miss Emma Reid, Miss Laura McKenzie and Dr Asgar Ali. Project management and resources support was provided by Dr Indrajit Chaudhari, Dr Nupur Roy, Dr Naresh Gill, Dr Chandramani Singh, Dr Neeraj Agarwal, Dr Sadhana Sharma, Dr Sridhar Srikantiah, Dr Prabhas Kumar Mishra and Mr Karthick Morchan. Support for managing the database system used during the project was provided by Miss Gala Garrod and Miss Anna Trett. Dr Geraldine Foster provided support in funding acquisition and field supervision. Dr Mike Coleman and Professor Janet Hemingway provided supervisorial support to Rinki on all aspects of the research.

5.1 INTRODUCTION

Indoor residual spraying (IRS) involves the application of insecticide formulations to the interior walls of houses, animal shelters and public buildings where people are at risk of transmission of insect borne

diseases. The organochlorine insecticide, dichlorodiphenyltrichloroethane (DDT) was introduced for vector control in 1946 (Mabaso, Sharp and Lengeler, 2004), before being used at scale during the Global Malaria Eradication Campaign between 1955-1969 (ME, 1966; World Health Organization, 2006b; Nájera, González-Silva and Alonso, 2011) resulting in the elimination of malaria from 37 countries.

Visceral leishmaniasis (VL) is caused by the parasite *Leishmania donovani* and in South Asia (Indian subcontinent) is transmitted by the bite of the female sand fly *Phlebotomus argentipes* (Singh, Pandey and Sundar, 2006; Joshi *et al.*, 2008). From 1953-1962 DDT-based IRS was carried out in India by the national malaria control programme. This had the secondary impact of controlling *P. argentipes* and almost eliminating VL (Deb *et al.*, 2018). After IRS for malaria ceased in VL endemic areas, DDT IRS was used intermittently to control VL outbreaks between 1977-1979 and 1992-1995 (Thakur, 2007; Muniaraj, 2014). Between 2004-2010 there were an estimated 200,000-400,000 new cases of VL annually (Alvar *et al.*, 2012), with 67% of these occurring in Bangladesh, India and Nepal. Today in India, 130 million people from 54 districts within the four endemic States of Bihar, Jharkhand, Uttar Pradesh and West Bengal remain at risk of VL. The World Health Organization (WHO) still estimates 300,000 annual cases of VL globally (World Health Organization, 2020c), however, only 16,970 cases were recorded in 2018 in the Global Health Observatory data repository (World Health Organization, 2020b), reflecting enhanced VL control measures.

In 2005, a tripartite agreement between Bangladesh, India, and Nepal was signed with the aim of eliminating VL and post–kala-azar dermal leishmaniasis as a public health problem *i.e.* to less than one case per 10,000 population by 2015 (World Health Organization, 2005). Elimination was attempted using a combination of vector control, rapid diagnosis and treatment of the disease (Singh, Pandey and Sundar, 2006; Singh *et al.*, 2011).

The VL elimination programme was planned in four phases: the preparatory phase which involved initiating improved case detection and biannual IRS; the attack phase where prevention and treatment activities were scaled up and monitoring was increased; the consolidation phase when the elimination target should be reached, and post elimination validation to maintain elimination, when surveillance is scaled-up to avoid resurgence (World Health Organization, 2012c). India aimed to reach the consolidation phase by 2015, but timescales were revised to 2017 and then 2020. Despite the disruption of the Coronavirus (COVID-19) global pandemic in 2020, IRS vector control activities have been maintained to reach the elimination target.

In elimination settings, an integrated vector management approach is ideal (World Health Organization, 2005), but there are limited data to demonstrate the impact of different vector control methods on VL transmission. A cluster randomized trial in Bangladesh, India, and Nepal demonstrated that insecticide-based IRS reduced the indoor abundance of *P. argentipes* by 72.4% in intervention clusters compared with controls; this effect was greater than the effect of environmental modification (42% reduction) or the use of long-lasting insecticide-treated nets (43.7% reduction) (Joshi *et al.*, 2009). Transmission models also suggest that IRS is capable of achieving VL elimination if sand fly abundance can be reduced by 67% (Stauch *et al.*, 2014). Hence, if case detection and treatment with effective drugs and effective IRS are combined, elimination of VL as a public health problem should be feasible (Singh, Pandey and Sundar, 2006; Singh *et al.*, 2011).

In India, to maximize the impact IRS is carried out in houses and cattle shelters as *P. argentipes* shows endophilic and exophagic behaviour (Dinesh *et al.*, 2001). Cattle sheds are included as *P. argentipes* collected outdoors and in cattle sheds using CDC light traps (Poché *et al.*, 2011) have predominantly fed on humans (Poché *et al.*, 2011, 2012). Based on previous success, India initially used DDT IRS, applying a wettable powder formulation at 1g/ m^2 with stirrup pumps. However, by 2013, progress towards the consolidation phase was limited (World Health Organization, 2005). Operationally relevant levels of resistance to DDT in *P. argentipes* and sub-optimal dose delivery of IRS were identified as key barriers to success (Coleman *et al.*, 2015). This prompted the National Vector Borne Disease Control Programme (NVBDCP) to switch to a pyrethroid insecticide, alpha-cypermethrin 5% wettable powder, to overcome resistance (Directorate National Vector Borne Disease Control Programme, 2016b) and to compression pumps (Hudson X-pert Sprayer) to improve the quality of IRS delivery, in accordance with WHO guidelines for IRS (World Health Organization, 2015).

To monitor the impact of vector control, the systematic entomological tracking of vector species and their characteristics is critical (World Health Organization, 2016a). WHO defines entomological surveillance as the regular, systematic collection, analysis and interpretation of entomological data for risk assessment, planning, implementation, monitoring and evaluation of vector control interventions with key indicators including the abundance of the vector species and insecticide resistance (World Health Organization, 2018, 2018). In India, this was undertaken in collaboration with the NVBDCP using routine sentinel surveillance from 2016 onwards; to assess the impact on disease burden, VL case incidence was tracked along with entomological indicators.

5.2 MATERIALS AND METHODS

5.2.1 Sentinel Sites

Eight sentinel sites in VL endemic areas were established: six in Bihar, one in Jharkhand and one in West Bengal. Each site had at least 1 new VL case per 10,000 persons per year at sub-district (block)

level. In the State of Bihar, the sites also represented ecologically diverse regions. Block selection was based on total reported VL case numbers, extracted from the 2015 district level IRS micro plan data. At the village level, criteria for selection included: VL case history for the previous three consecutive years, appropriate infrastructure to allow year-round village access and absence of additional planned field research activities. Of the villages that met the selection criteria, four IRS villages per sentinel site were selected using a random number generator in Microsoft Excel. A further two villages with no history of IRS or VL cases for the previous five years were also selected per sentinel site using the same random number generator method, to monitor any social or seasonal effects on entomological indicators that are unrelated to IRS.

5.2.2 Indoor residual spray routine coverage data

Routine coverage data by IRS round for 2016–2019 for the sentinel site villages were obtained from spray supervisor registers, held at the District Malaria Office. Where possible, data on the rooms, cattle sheds and verandas targeted for treatment and whether these were sprayed, locked or refused were digitised. Coverage rates (sprayed, locked and refused) were calculated for rooms and cattle sheds.

5.2.3 Indoor residual spray survey coverage data

IRS coverage data was obtained from community-based cross-sectional studies conducted biannually between March—June and July—September, in the VL sentinel districts of Bihar and Jharkhand (West Bengal was not included in this survey). As part of a survey to assess IRS quality, led by CARE India, sample size of 800 households per district were targeted for each spray round in each of these districts assuming a 95% confidence level, 5% absolute precision and 50% expected coverage based on the most conservative measure and accounting for any cluster effects. A subset of this data, relating to the sentinel sites was extracted and used for analysis.

In each district, 40 villages were selected from the operational IRS plans using Probability Proportional to Size. From each village 20 houses were systematically selected with a random start (The Index/Starting point was selected randomly from Anganwadi's household survey register, where each house was numerated, using a random number table). Interviews were carried out manually or using Computer Assisted Personal Interview (CAPI) tools in the local language to assess if houses and cattle sheds had been completely sprayed, partially sprayed or not sprayed. The sampling was designed to provide estimates of IRS coverage for the villages targeted for spraying in each IRS round in each district. Surveys were typically completed within a month of completion of each round of IRS.

5.2.4 Quality assurance

Samples for quality assurance were collected from all 8 sentinel sites from 2017 to 2019. Surveys were conducted in rooms within houses where *P. argentipes* abundance monitoring was ongoing.

To determine the concentration of alpha-cypermethrin delivered to walls during IRS, 5cm^2 Whatman Grade 1 filter papers were affixed onto all four walls of the room prior to IRS, as described by WHO [26]. Single filter papers were affixed between 2–4 ft from the ground, on all four walls within the bedroom and stored at -4°C until analysis following IRS activities.

The concentration of insecticide present on the filter papers was determined using high performance liquid chromatography (HPLC). All filter papers were cut into pieces of ~1 cm^2. Five ml of a heptane/1-propoanol mixture (9:1) containing 100 µg of the internal standard dicyclohexyl phthalate (DCP) was added, and samples were sonicated for 15 min to extract the alpha-cypermethrinin. The insecticide extract (1ml) was transferred to a clean glass tube and evaporated to dryness at 60°C. One ml of acetonitrile was added, and the mixture was vortexed for 1 min to mix.

HPLC analysis was performed by injection of 20-µL aliquots of extract on a reverse-phase Hypersil GOLD C18 column (75 Å, 250 × 4.6 mm, 5-µm particle size; Thermo Scientific) at 23–25°C. A mobile phase of acetonitrile/water (70:30) was used at a flow rate of 1 mL·min−1 to separate alpha-cypermethrin and DCP. The quantities of alpha-cypermethrin and DCP were calculated from standard curves established by known concentrations of authenticated standards. Peaks were detected at 232 nm with the Agilent 1260 Infinity Quaternary LC system, model G1311C detector (Agilent) and were analysed with Agilent OpenLAB CDS software.

Final alpha-cypermethrin content in grams per square meter was estimated using the following equations:

$$B = (P/V)xDx(100/E)xC$$

Where alpha-cypermethrin (g/m2) (A) = ((B/S)x10,000)/1,000,000, B = alpha-cypermethrin (µg/25cm), P = peak area, V = slope value, D = dilution factor (5), E = extraction efficiency (100%), C = DCP correction factor, S = surface area of filter paper (25cm^2).

HPLC results were compared with the intended IRS target alpha-cypermethrin concentration on the wall of 25.0mg/m^2. A 20% cut-off threshold was used to classify results whereby a concentration of less than 20.0 mg/m^2 was considered an under-spray, a range of 20.0–30.0 mg/m^2 was considered within the target range, and a concentration of greater than 30mg/m^2 was considered an overspray.

5.2.5 Insecticide susceptibility assays

Female *P. argentipes* were collected using mouth aspirators inside houses, verandas, and cattle sheds. Collected sand flies were exposed to alpha-cypermethrin (0.05%, 0.065% and 1%), bendiocarb (0.1%), deltamethrin (0.05%), DDT (4%) or malathion (5%) using WHO-impregnated filter papers following the WHO susceptibility test procedures (World Health Organization, 2013). Mortality was recorded after 24 hours. Controls were performed for each test using appropriate papers, and Abbott's formula was applied where necessary (Abbott, 1987).

5.2.6 *Phlebotomus argentipes* abundance

Year-round *P. argentipes* abundance was monitored using CDC light traps operating in 15 randomly selected houses in each village over a period of two consecutive nights (6:00 PM to 6:00 AM) on a bi-monthly basis. The light traps were hung in the corner of a bedroom and optimally positioned 15 cm away from the wall and 5 cm above ground. All sand flies were identified to species level by morphological criteria from established taxonomic keys (Kalra and Bang, 1988).

Abundance was analysed by a generalised additive model (GAM) being fitted to the data aggregated to the village-level and monthly time scale to model the changes in sandfly abundance over time, accounting for effect of IRS status (IRS or Non-IRS) of the village. Seasonal effects were modelled as a cubic regression spline, whereas long-term temporal trends were modelled using thin plate regression splines. A first order autoregressive component (AR(1)) was included in the model to account for temporal correlation.

5.2.7 Identification of *L. donovani* in *P. argentipes*

L. donovani parasite kinetoplastid DNA (kDNA) in *P. argentipes* sand flies was detected by RTPCR (Adams *et al.*, 2018). Analysis was initially done on pooled sand fly DNA (maximum of 8 sand flies per pool), any positive pooled results were investigated further at the individual sand fly level.

5.2.8 Case data for sentinel sites

Total annual case data (2016 to 2019) for the blocks containing the sentinel sites was extracted from the Kala-Azar Management Information System (KAMIS) database. Population calculated using Government of India 2011 Census (Directorate of Economics & Statistics (Bihar Patna), 2011) and population projected using the formula: Population Final = Population initial ((1+(growth rate/100))^(time in years)). In the absence of open-access data on the population at risk of VL within Bihar, the total state population was used as the denominator to calculate incidence per 10,000 [7]. Case incidence rate per 10,000 = (total number new cases/persons at risk) x10,000.

5.3 RESULTS

5.3.1 Sentinel Sites

The eight VL sentinel sites across Bihar, Jharkhand and West Bengal were established in a phased approach starting with Muzaffarpur and Samastipur in April 2016 and ending Darjeeling in November 2017 (Table 12).Figure 17 shows the location of the sites (Darjeeling, East Champaran, Godda, Gopalganj, Katihar, Muzaffarpur, Purnia and Samastipur). Within each sentinel site, a total of four IRS villages and 2 non-IRS villages were randomly selected and monitored.

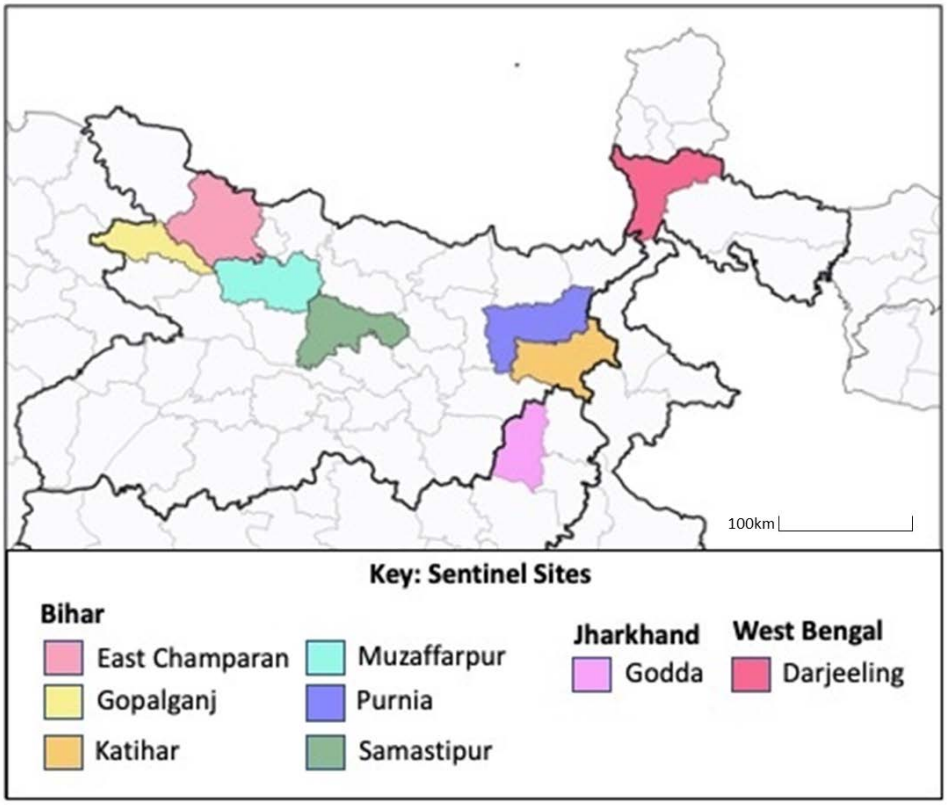

Figure 19: Map of the three VL endemic areas in India with the sentinel site districts labelled.

Where possible, this ratio of non-IRS to IRS villages was maintained. However, over the three years of monitoring, four changes in IRS status occurred in response to the emergence of VL cases in non-IRS villages (Table 12). In Purnia, both non-IRS villages were sprayed within 12 months of collections starting, and in 2019, after a spike in VL cases in Gopalganj, both non-IRS villages were sprayed. Randomly selected houses within the sentinel sites remained fixed to enable longitudinal monitoring unless house owners opted to leave the study. Households opting to leave were replaced with houses selected using the same random selection methodology

Table 12: Location of sentinel site villages and dates of entomological collections

District	Block	Village	IRS status	Collection dates (mm/yy)	Comments
Darjeeling	Phansidewa	Madhavita	IRS	11/17 to 12/19	
		Moonee div.	IRS	11/17 to 12/19	
		Motidhar T.E	IRS	11/17 to 12/19	
		Taipoo T.E	IRS	11/17 to 12/19	
		Kalamgachh	Non-IRS	11/17 to 12/19	
		Piapukur	Non-IRS	11/17 to 12/19	
East Champaran	Turkauliya	Jaisinghpur north	IRS	10/17 to 12/19	
		madhopur	IRS	10/17 to 12/19	
		Madhumalat	IRS	10/17 to 12/19	
		Mathurapur	IRS	10/17 to 12/19	
		Bijulpur	Non-IRS	10/17 to 12/19	
		Chainpur	Non-IRS	10/17 to 12/19	
Godda	Poraiya Haat	Bhatonda	IRS	01/17 to 12/19	
		Gumma	IRS	01/17 to 12/19	
		Kathon	IRS	01/17 to 12/19	
		Sakri	IRS	01/17 to 12/19	
		Baxara	Non-IRS	01/17 to 12/19	
		Birniya	Non-IRS	01/17 to 12/19	
Gopalganj	Barauli	Barauli	IRS	10/16 to 12/19	
		Kalyanpurmathiya	IRS	10/16 to 12/19	
		Rupanchap	IRS	10/16 to 12/19	
		Sadaua	IRS	10/16 to 12/19	
		Sarar	Non-IRS	10/16 to 06/19	sprayed in response to spike in cases
			IRS	06/19 to12/19	
		Jokaha	Non-IRS	10/16 to 04/19 11/19 to12/19	sprayed in response to spike in cases
			IRS	04/19 to 11/19	
Katihar	Barari	Balua	IRS	10/16 to 12/19	
		Kajra	IRS	10/16 to 12/19	
		Kawar-kothi	IRS	10/16 to 12/19	
		Siwana	IRS	10/16 to 12/19	
		Ghuski	Non-IRS	10/16 to 12/19	
		Milik tola	Non-IRS	10/16 to 12/19	
Muzaffarpur	Minapur	Alineora	IRS	04/16 to 03/18	
			Non-IRS	04/18 to 12/19	
		Bajarmuriya	IRS	04/18 to 12/19	new village added
		Chandparna	IRS	04/16 to 12/19	
		Maksoodpur	IRS	04/16 to 03/18	
			Non-IRS	12/18 to 12/19	
		Minapur	IRS	04/16 to 12/19	
		Bahwal	Non-IRS	04/16 to 12/19	
		Chakimaad	Non-IRS	04/16 to 12/19	
Purnia	Dhamdaha	Bishanpur	IRS	10/16 to 12/19	
		Dhamdaha uttar	IRS	04/16 to 12/19	
		Dharharjamuniya	IRS	04/16 to 12/19	
		Kajra	IRS	04/16 to 12/19	
		Kukron	Non-IRS	10/16 to 11/17	
			IRS	12/17 to 12/19	
		Parasmani	Non-IRS	10/16 to 12/16	
			IRS	01/17 to 12/19	
Samastipur	Warishnagar	Balahi	IRS	04/16 to 12/19	

District	Block	Village	IRS status	Collection dates (mm/yy)	Comments
		Dhanhar	IRS	04/16 to 12/19	
		Kusaiya	IRS	04/16 to 12/19	
		Rahua east	IRS	04/16 to 03/17	
			Non-IRS	03/17 to 12/19	
		Chandauli	Non-IRS	04/16 to 12/19	
		Kashor	Non-IRS	04/16 to 12/19	

5.3.2 Indoor residual spraying data

In order to be effective IRS needs to be applied at the optimal time of year, with high coverage of all targeted structures at an accurate dosage (World Health Organization and TDR, 2010). In the current programme, biannual spraying is targeted in March and August, with spray operators reporting over 80% coverage of households and cattle sheds covered with alpha-cypermethrin at 25mg/m^2 IRS.

5.3.3 IRS coverage as reported by spray teams

IRS coverage data was provided by NVBDCP at the spray team level from 2017–2019 for each of the sentinel sites (Figure 18).

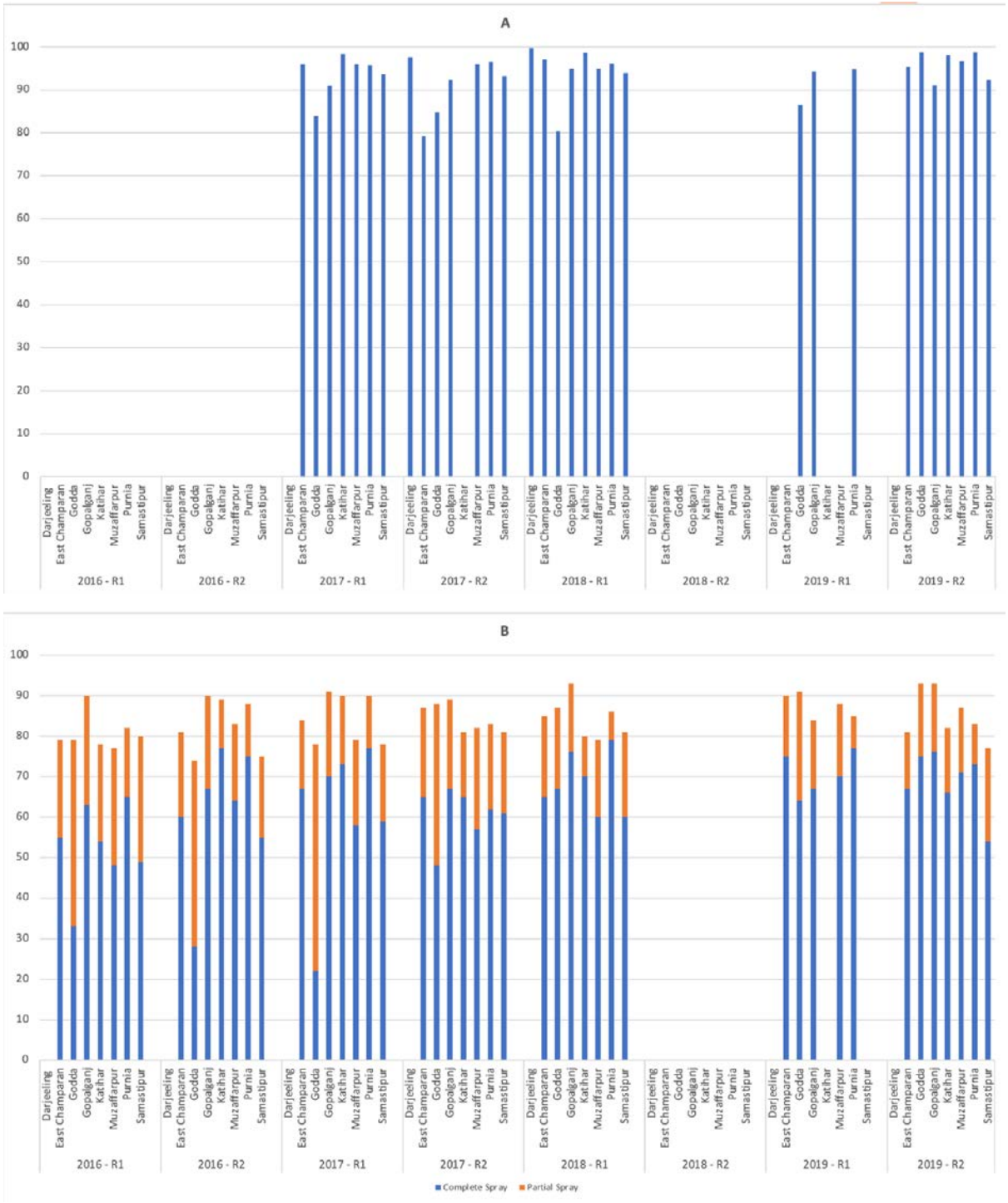

Figure 20: Percentage of household structures that were completely sprayed in each IRS round as reported by; A spray operators in all 8 sites, and B sentinel sites surveys.

R1 = Round one R2 = Round two. Solid colours represent complete spray and lined colours partial spray.

Due to operational issues only one round of IRS was undertaken in 2018. Data obtained from the spray registers completed by spray teams predominantly showed high levels of IRS coverage for household rooms (79.3–99.7%) and cattle sheds (68.3–100%) across all spray rounds (Table 13).

Table 13: Indoor residual spraying by district that the sentinel sites are located as reported by spray operators.

Year	IRS Round	District	Rooms								
			Sprayed (Complete)		Sprayed (Partial)		Refused		Locked		Total
			%	n	%	n	%	n	%	n	
2017	1	East Champaran	95.97	15,335	0.00	0	2.62	419	1.41	225.00	15,979
		Godda	83.86	6,589	2.02	159	14.11	1,109	0.00	0.00	7,857
		Gopalganj	90.92	2,114	0.00	0	9.08	211	0.00	0.00	2,325
		Katihar	98.36	9,548	0.00	0	1.04	101	0.60	58.00	9,707
		Muzaffarpur	95.92	12,701	0.00	0	2.48	328	1.60	212.00	13,241
		Purnia	95.70	10,619	0.00	0	2.53	281	1.77	196.00	11,096
		Samastipur	93.61	10,826	0.00	0	4.23	489	2.16	250.00	11,565
	2	Darjeeling	97.65	1,081	0.00	0	1.45	16	0.90	10.00	1,107
		East Champaran	79.35	1,975	0.00	0	12.86	320	7.79	194.00	2,489
		Godda	84.76	6,466	0.00	0	9.39	716	5.86	447.00	7,629
		Gopalganj	92.41	6,059	0.00	0	7.59	498	0.00	0.00	6,557
		Muzaffarpur	96.00	12,034	0.00	0	1.78	223	2.23	279.00	12,536
		Purnia	96.44	14,931	0.00	0	2.40	372	1.16	179.00	15,482
		Samastipur	93.11	13,061	0.00	0	3.64	511	3.25	456.00	14,028
2018	1	Darjeeling	99.63	4,838	0.00	0	0.08	4	0.29	14.00	4,856
		East Champaran	96.94	13,549	0.00	0	2.15	301	0.90	126.00	13,976
		Godda	80.49	5,404	0.00	0	9.47	636	10.04	674.00	6,714
		Gopalganj	94.99	7,074	0.00	0	5.00	372	0.01	1.00	7,447
		Katihar	98.67	5,413	0.00	0	0.86	47	0.47	26.00	5,486
		Muzaffarpur	94.88	6,469	0.00	0	1.54	105	3.58	244.00	6,818
		Purnia	96.05	14,576	0.00	0	2.43	369	1.52	230.00	15,175
		Samastipur	93.90	12,445	0.00	0	3.45	457	2.65	351.00	13,253

2019	**1**	Godda	86.44	7,169	0.00	0	7.99	663	5.57	462.00	8,294
		Gopalganj	94.35	5,089	0.00	0	3.13	169	2.52	136.00	5,394
		Purnia	94.85	17,882	0.00	0	3.18	600	1.97	371.00	18,853
	2	East Champaran	95.27	16,088	0.00	0	1.53	259	3.19	539.00	16,886
		Godda	87.63	6,985	0.00	0	7.39	589	4.98	397.00	7,971
		Gopalganj	91.08	5,994	0.00	0	4.88	321	4.04	266.00	6,581
		Katihar	98.20	3,378	0.00	0	0.99	34	0.81	28.00	3,440
		Muzaffarpur	96.74	9,994	0.00	0	0.87	90	2.39	247.00	10,331
		Purnia	98.79	9,153	0.00	0	0.17	16	1.04	96.00	9,265
		Samastipur	92.40	10,781	0.00	0	4.58	534	3.03	353.00	11,668

5.3.4 IRS coverage as measured from survey data

The household surveys indicated much lower complete house IRS coverage values (28 to 79%) than self-reported by the spray teams. The IRS coverage survey data for the sentinel sites located in Bihar and Jharkhand from 2016–2019 indicates that the WHO recommended minimum 80% coverage complete coverage for successful IRS (World Health Organization, 2015) was not met across all sentinel sites, although the 80% target was achieved if household bedroom coverage data, where most transmission occurs is considered. In 2016, coverage for complete spray ranged from 28% in Godda, to 77% in Katihar. By 2019, an improvement in IRS coverage was seen, with the minimum coverage (complete spray) achieved in in Samastipur (54%) and the maximum coverage in Purnia (77%). In Bihar, consistently low-level of complete coverage over the four years was observed in Samastipur (49–61%). The greatest improvement in spray complete IRS coverage was observed in Godda, Jharkhand where in 2016, 28% complete coverage was reported, which increased to 75% in 2019 (Figure 18). The 2019 household surveys reported a much higher complete coverage (54–77%) suggesting an overall improvement in the spray programme that can be seen in for each sentinel site in Figure 18. However, if partial spray coverage is included the percentage coverage ranges from 74% to 93% (Table 14).

Table 14: Indoor residual spray coverage data from household surveys.

Year	IRS Round	District	Cattle sheds								
			Sprayed (Complete)		Sprayed (Partial)		Refused			Locked	Total
			%	n	%	n	%	n	%	n	
2017	**1**	East Champaran	98.44	2,706	0.00	0	1.09	30	0.47	13	2,749
		Godda	No Data		No Data		No Data		No Data		0
		Gopalganj	98.63	432	0.00	0	1.37	6	0.00	0	438
		Katihar	99.83	1,199	0.00	0	0.00	0	0.17	2	1,201
		Muzaffarpur	97.24	3,587	0.00	0	1.41	52	1.36	50	3,689
		Purnia	98.39	2,378	0.00	0	0.46	11	1.16	28	2,417
		Samastipur	97.44	2,322	0.00	0	1.64	39	0.92	22	2,383
	2	Darjeeling	100.00	50	0.00	0	0.00		0.00		50
		East Champaran	68.31	194	0.00	0	27.46	78	4.23	12	284
		Godda	No Data		No Data		No Data		No Data		0
		Gopalganj	99.29	1,403	0.00	0	0.71	10	0.00	0	1,413
		Muzaffarpur	97.33	3,131	0.00	0	1.15	37	1.52	49	3,217
		Purnia	97.76	3,406	0.00	0	1.18	41	1.06	37	3,484
		Samastipur	96.95	2,096	0.00	0	1.62	35	1.43	31	2,162
2018	**1**	Darjeeling	100.00	200	0.00	0	0.00	0	0.00	0	200
		East Champaran	98.95	3,006	0.00	0	0.89	27	0.16	5	3,038
		Godda	No Data		No Data		No Data		No Data		0
		Gopalganj	99.82	1,666	0.00	0	0.18	3	0.00	0	1,669
		Katihar	100.00	715	0.00	0	0.00	0	0.00	0	715
		Muzaffarpur	95.96	1,354	0.00	0	1.77	25	2.27	32	1,411
		Purnia	97.59	2,920	0.00	0	1.37	41	1.04	31	2,992
		Samastipur	95.99	1,770	0.00	0	2.77	51	1.25	23	1,844
2019	**1**	Godda	No Data		No Data		No Data		No Data		0
		Gopalganj	95.53	982	0.00	0	1.95	20	2.53	26	1,028
		Purnia	96.72	2,772	0.00	0	1.64	47	1.64	47	2,866

Year	IRS Round	District	Cattle sheds								
			Sprayed (Complete)		Sprayed (Partial)		Refused			Locked	Total
			%	n	%	n	%	n	%	n	
	2	East Champaran	95.89	3,706	0.00	0	1.60	62	2.51	97	3,865
		Godda	No Data		No Data		No Data		No Data		0
		Gopalganj	99.12	674	0.00	0	0.44	3	0.44	3	680
		Katihar	97.48	310	0.00	0	0.94	3	1.57	5	318
		Muzaffarpur	97.88	2,171	0.00	0	0.77	17	1.35	30	2,218
		Purnia	99.07	1,590	0.00	0	0.37	6	0.56	9	1,605
		Samastipur	95.01	1,655	0.00	0	2.35	41	2.64	46	1,742

5.3.5 Quality assurance (QA)

A total of 642 houses that received IRS across the eight sites were included in the QA surveys. Four filter papers per wall were affixed in the bedroom prior to IRS and recovered afterwards. A total of 2,992 Whatman filter papers were retrieved and analysed by HPLC over three years (2017–2019). In 2017, all 2,140 filter papers collected from field surveys were analysed. In subsequent years a minimum random sample of 20% of houses from each sentinel site were analysed. The total number of filter papers retrieved per year varied due to change in IRS status of some of the villages, or programmatic decisions to spray villages where there was no VL history. In addition, upon retrieval some filter papers were found to be missing from the affixed position and therefore no sample was available.

Of the 2,992 filter papers tested from 2017 to 2019 only 15.4% had been sprayed at the target dose (25mg2 ± 10% range 20-30mg/m2). The best IRS quality was observed in Samastipur with 25.71% of filter papers on target during round one of IRS in 2018 (Figure 19).

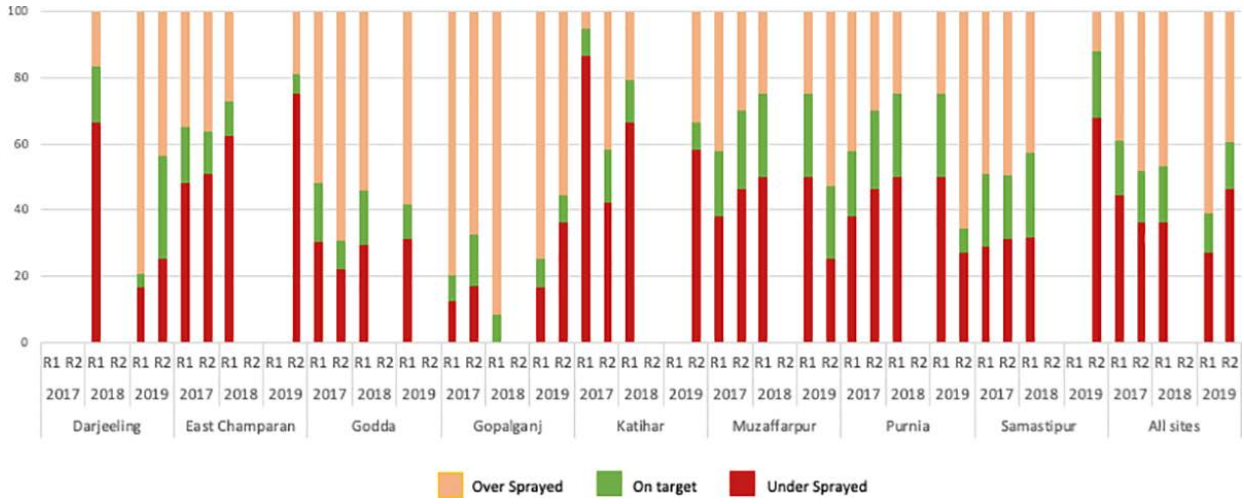

Figure 21: Percentage of household structures that received the correct dose, overdose and under dose of alpha-cypermethrin.

R1-Round 1; R2-Round 2; % over sprayed- >30 mg/m^2; % on target-20-30mg/m^2; % under sprayed—<20 mg/m^2 alpha-cypermethrin.

High levels of under spraying (86.67%) were observed in Katihar during 2017's first round of IRS: spray performance improved in subsequent years with 58.33% of filter papers analysed under sprayed in 2019 round 2. Consistently high levels of over-spraying were observed in Gopalganj for all three years of filter paper analysis (55.56–91.67%). In East Champaran the quality of spraying declined annually from 48.3% under spray in round 1 of 2017 to 75% under spray in round 2 of 2019.

Considering all the filter papers collected from 2017–2019 the average concentration of insecticide by year, irrespective of geography was relatively consistent over the time period (2017: 40.03mg/m^2, 2018: 41.64mg/m^2 and 37.92mg/m^2). The average doses on the filter papers were 1.6 times higher than the target concentration of insecticide (25mg/m^2) in 2018 this was 1.67 times and 2019 1.52 times. This overdosing may in part be due to IRS operators over spraying the filter papers. The highest level of over spray was detected in Muzaffarpur in 2018 (657.1mg/m^2). During the 2018 IRS campaign the target dose range (20-30mg/m^2) was achieved in surveys from three of the eight districts.

5.3.6 Susceptibility Assays

The wild caught female *P. argentipes* mortality ranged from 39.9–66.7% for 4% DDT after exposure to WHO insecticide impregnated. Minimal resistance was detected to alpha-cypermethrin at any of the three concentrations tested (0.05%, 0.0675% and 1%) with mortality ranging from 97.6–100% during this period. No resistance was detected to the other insecticides tested (Table 15).

Table 15: P. argentipes mortality over time for a range of insecticides in WHO susceptibility tests.

Insecticide	2016		2017		2018		2019	
	%mort	*n*	%mort	*N*	%mort	*N*	%mort	*n*
Alpha-cypermethrin 0.05%	99.62	527	97.60	1126	99.87	4566	99.47	2340
Alpha-cypermethrin 0.0675%	97.87	947	99.01	1997	99.49	4219	98.68	2424
Alpha-cypermethrin 0.1%	100	214	97.99	1280	99.71	3236	99.74	2920
Bendiocarb 0.1%			98.73	1007	98.49	3063	100	745
Deltamethrin 0.05%			99.63	1142	98.93	2969	99.93	1597
DDT 4%	66.67	63	49.55	1351	44.35	2316	39.90	1377
Malathion 5%			98.86	1100	99.96	2737	100	905

When considering susceptibility to insecticides at a sentinel site level (Table 16), the lowest level of mortality after exposure to 4% DDT papers was observed in Samastipur in 2018 (24.53%) whilst the highest level of mortality was observed in 2017 in Muzaffarpur (76.16%). Interesting, a low level of mortality to the diagnostic dose of alpha-cypermethrin (0.05%) was observed in Godda in 2017 (87.48%), however in subsequent years 100% mortality was observed. Similar trends were also seen in Godda for malathion 5% and bendiocarb 0.1% where 92.67% and 92.3% mortality was observed respectively in 2017. In 2019 however, 100% mortality was recorded for both insecticides in Godda. Finally, reduced mortality after exposure to 0.05% deltamethrin was observed in Samastipur in 2019.

Table 16: Susceptibility to insecticides at sentinel site level

Insecticide	**Year**	**Corrected mortality (%) and number of P.argentipes exposed (n)**							
		Darjeeling	**East Champaran**	**Godda**	**Gopalganj**	**Katihar**	**Muzaffarpur**	**Purnia**	**Samastipur**
DDT-4%	**2016**	No Data	No Data	No Data	No Data	No Data	66.67 (n=63)	No Data	No Data
	2017	0 (n=)	43.81 (n=396)	18.83 (n=42)	41.29 (n=167)	26.99 (n=163)	76.16 (n=172)	42.53 (n=87)	63.89 (n=324)
	2018	28.75 (n=240)	49.81 (n=528)	35.8 (n=537)	55 (n=80)	67.63 (n=173)	65.66 (n=332)	36.65 (n=161)	24.53 (n=265)
	2019	No Data	57.03 (n=526)	20.62 (n=516)	No Data	No Data	42.69 (n=335)	No Data	No Data
Alpha-cypermethrin-0.05%	**2016**	No Data	99.33 (n=298)	No Data	100 (n=22)	No Data	100 (n=207)	No Data	No Data
	2017	100 (n=20)	100 (n=176)	87.48 (n=182)	100 (n=164)	96.93 (n=163)	100 (n=176)	97.56 (n=82)	100 (n=163)
	2018	100 (n=360)	100 (n=528)	100 (n=998)	100 (n=413)	99.6 (n=250)	99.48 (n=576)	100 (n=564)	99.77 (n=877)
	2019	95.83 (n=240)	100 (n=492)	100 (n=495)	100 (n=338)	97.66 (n=171)	100 (n=588)	98.77 (n=162)	94.47 (n=420)
Alpha-cypermethrin-0.0675%	**2016**	No Data	100 (n=406)	No Data	100 (n=351)	90.06 (n=161)	No Data	82.52 (n=29)	No Data
	2017	95 (n=40)	100 (n=484)	96.94 (n=420)	100 (n=354)	99.03 (n=206)	100 (n=167)	97.92 (n=144)	100 (n=182)

Insecticide	Year	Corrected mortality (%) and number of P.argentipes exposed (n)							
		Darjeeling	East Champaran	Godda	Gopalganj	Katihar	Muzaffarpur	Purnia	Samastipur
	2018	99.5 (n=400)	100 (n=528)	97.86 (n=537)	100 (n=415)	100 (n=258)	100 (n=577)	98.96 (n=577)	99.78 (n=927)
	2019	98.75 (n=320)	100 (n=520)	96.32 (n=511)	99.7 (n=333)	100 (n=82)	100 (n=571)	98.76 (n=242)	97.17 (n=258)
Alpha-cypermethrin-0.1%	2016	No Data	No Data	No Data	No Data	No Data	100 (n=168)	No Data	100 (n=46)
	2017	100 (n=40)	100 (n=176)	85.57 (n=178)	100 (n=166)	100 (n=164)	100 (n=167)	100 (n=222)	100 (n=167)
	2018	100 (n=444)	100 (n=496)	100 (n=508)	100 (n=429)	99.16 (n=357)	100 (n=580)	99.39 (n=163)	97.92 (n=259)
	2019	100 (n=400)	100 (n=518)	99.12 (n=501)	100 (n=327)	98.83 (n=171)	99.81 (n=582)	100 (n=164)	100 (n=257)
Bendiocarb-0.1%	2017	No Data	100 (n=176)	92.3 (n=166)	100 (n=160)	100 (n=86)	100 (n=164)	100 (n=90)	100 (n=165)
	2018	100 (n=324)	100 (n=528)	91.69 (n=556)	100 (n=404)	100 (n=175)	100 (n=570)	100 (n=164)	100 (n=342)
	2019	100 (n=80)	100 (n=528)	100 (n=491)	100 (n=338)	No Data	100 (n=577)	100 (n=163)	100 (n=177)
Deltamethrin-0.05%	2017	No Data	100 (n=132)	98.44 (n=292)	100 (n=167)	100 (n=168)	100 (n=164)	100 (n=149)	100 (n=170)
	2018	99.52 (n=416)	100 (n=528)	94.88 (n=542)	100 (n=323)	No Data	100 (n=580)	98.77 (n=163)	100 (n=169)
	2019	100 (n=80)	100 (n=528)	99.77 (n=486)	100 (n=329)	No Data	100 (n=583)	98.77 (n=162)	95.7 (n=172)
Malathion-5%	2017	No Data	100 (n=176)	92.67 (n=171)	100 (n=165)	100 (n=165)	100 (n=172)	100 (n=82)	100 (n=169)
	2018	100 (n=240)	100 (n=528)	99.82 (n=541)	100 (n=344)	100 (n=169)	100 (n=577)	100 (n=165)	100 (n=173)
	2019	No Data	100 (n=528)	100 (n=485)	100 (n=327)	No Data	100 (n=500)	100 (n=164)	98.21 (n=168)

5.3.7 *Phlebotomus argentipes* abundance

Over the three and a half years a total of 102,951 CDC light trap collections were performed in the eight sentinel sites, from which a total of 91,571 female *P. argentipes* sand flies were identified. In IRS villages, a total of 62,384 (69,450 collections) *P. argentipes* sand flies were collected. In comparison a total of 29,187 (33,501 collections) *P. argentipes* sand flies were collected in non-IRS villages.

The peak period for sand fly abundance irrespective of village IRS status was between June and September with a peak abundance reaching 2.92 (July-2016), 3.00 (July-2017), 1.81 (July-2018), 1.26

(September-2019) sand flies per trap per night in IRS villages and 3.38 (July-2016), 2.52 (July-2017), 2.05 (June-2018) and 1.77 (July-2019) sand flies per trap per night in Non-IRS villages.

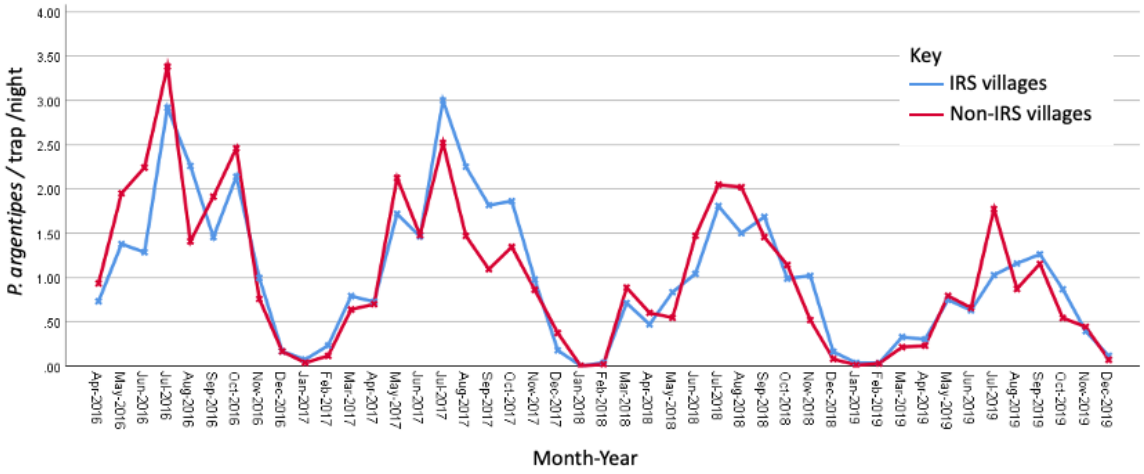

Figure 22: Aggregated P. argentipes abundance collected in all sentinel sites for IRS and non-IRS villages.

Throughout the study period a general annual decline in *P. argentipes* abundance was observed (log relative risk= -0.01684, P=0.0542) (Table 17). Previously abundance has been reported at 4.0 to 5.5 *P. argentipes*/trap/night for 2014 (Coleman *et al.*, 2015), in IRS villages, and here we report a reduction to 0.75 *P. argentipes*/trap/night for the same time period by 2019.

Table 17: Generalised Additive Model Analysis result of P. argentipes abundance data from April 2016 to December 2019.

GAM Analysis			
Parameter	**log-RR**	**95% CI**	**P-value**
Month (Linear)	-0.0168	0.9668-1.0000	0.0542
IRS	-0.0036	0.8194-1.4392	0.5086

Figure 20 shows the monthly trends in *P. argentipes* abundance aggregated by village-level IRS status. On fitting a GAM to the village-level monthly abundance data, it was noted that the smoothed long-term temporal trend was very close to linear, therefore time was included as a linear term rather than a smoothed function in the model. In the resulting model both the long-term temporal trend and the smoothed seasonal trend (s(month)) were significant, whereas IRS status was not significant (p=0.5086). This indicates that after accounting for seasonal trends (Figure 20), a general decline in *P. argentipes* abundance was observed over the study period (log-relative risk=-0.0168, p=0.0542), with no discernible difference in trends observed in IRS and non-IRS villages (p=0.5679). This along with the

GAM analysis suggests that there was no seasonal or social activity (e.g., lime plastering of walls) impacting IRS (World Health Organization, 2010).

5.3.8 Identification of *L. donovani* in *P. argentipes* sand flies

A total of 14,775 *P. argentipes* were assessed for the presence of *L. donovani* across all IRS sentinel site villages. Only 4 *P. argentipes* sand flies from East Champaran were positive (Table 18), suggesting that there is a very low active transmission of VL at the sentinel sites.

Table 18: Number of P. argentipes with positive L. donovani detection at each sentinel site by year.

	2017		2018		2019		**Total**	
	N. Tested	N. +ve	N. Tested	N. +ve	N. Tested	N. +ve	N. Tested	N. +ve
Darjeeling	54	0	271	0	544	0	869	0
East Champaran	991	3	704	1	936	0	2361	4
Godda	1056	0	723	0	892	0	2631	0
Gopalganj	1080	0	702	0	456	0	2671	0
Katihar	893	0	207	0	586	0	1686	0
Muzaffarpur	699	0	817	0	484	0	2000	0
Purnia	505	0	253	0	632	0	1390	0
Samastipur	191	0	562	0	537	0	1290	0
Total	5469	3	4239	1	5067	0	**14775**	4

5.3.9 Case data for sentinel sites

From 2016 to 2019 a total of 764 VL cases were reported in the blocks with the sentinel sites. A steady decline in incidence was observed in all blocks, apart from Darjeeling, Gopalganj and Samastipur, where an increase was seen in 2018. The highest incidence of 3.63 cases per 10,000 was observed in 2018 in Godda which along with Gopalganj consistently had high case numbers. Other than Gopalganj, the threshold for VL elimination of an incidence less than 1/1000 people at the block level, was reached and sustained from 2018 onwards in the sentinel site blocks, with the lowest incidence of 0.042 being observed in Samastipur in 2019, shown in Table 19. When aggregated there is a decline in incidence from 1.16 VL cases per 10,000 in 2016 to 0.51 VL cases per 10,000 in 2019, which is below the elimination target.

Table 19: Case data for blocks associated with sentinel sites from KAMIS.

Sentinel Site	2016			2017			2018			2019		
	Pop.	C.	Inc.	Pop.	C.	Inc.	Pop.	C.	Inc.	Pop.	C.	Inc.
Darjeeling - Phansidewa Block	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
East Champaran - Turkaulia Block	191,010	15	0.78	193,040	7	0.363	195,042	7	0.359	195,042	4	0.205
Godda - Poraiyahat Block	ND	ND	ND	ND	ND	ND	202,745	7	0.345	202,745	4	0.197
Gopalganj - Barauli Block	235,100	63	2.68	237,598	36	1.515	240,063	84	3.499	240,063	51	2.124
Katihar - Barari Block	302,222	17	0.56	305434	8	0.262	308602	14	0.454	308,602	11	0.356
Muzaffarpur -Minapur Block	361,044	45	1.24	364,880	27	0.740	368,665	32	0.868	368,665	17	0.461
Purnia - Dhamdaha Block	305,085	33	1.082	308,326	46	1.492	311,525	25	0.803	311,525	10	0.321
Samastipur - Warishnagar Block	228,877	9	0.393	231,309	5	0.216	233,708	16	0.685	233,708	1	0.043
Total	**1,623,377**	**181**	**1.11**	**1,640,587**	**145**	**0.88**	**1,860,350**	**186**	**0.89**	**1,879,648**	**98**	**0.52**

Pop. Population calculated using Government of India 2011 Census (Directorate of Economics & Statistics (Bihar Patna), 2011) and population projected using the formula: Population Final = Population initial ((1+(growth rate/100))^(time in years))

5.3.10 Limitations

As the surveillance data collection presented here was initiated 10-years after the beginning of the VL elimination programme there is no true-baseline available for comparison. This is also an observational study based on the 8 VL surveillance sites for data collection, which represents only 8 of the 600 blocks that are endemic for VL. While analysis of trends has been limited to these sites, the assumption of reduced incidence across all blocks is due to the overall impact of enhanced case detection, treatment, and vector control.

5.4 DISCUSSION

VL elimination as a public health problem is defined as reducing the annual incidence to <1 case per 10,000 people at the block level (Singh, Pandey and Sundar, 2006). In 2002 the National Health Policy of the Government of India was to eliminate VL from the region by 2010 (Joshi *et al.*, 2008; Nájera,

González-Silva and Alonso, 2011). In 2005, the governments of Bangladesh, India, and Nepal developed a strategic regional framework to eliminate VL as a public health problem by 2015 (Joshi *et al.*, 2008; Nájera, González-Silva and Alonso, 2011). This original target assumed that a VL vaccine in late-stage development could be incorporated into the elimination efforts. When the vaccine programme failed, the elimination target year was revised to 2017 and then to 2020 (Mabaso, Sharp and Lengeler, 2004; Singh, Pandey and Sundar, 2006).

The main strategies recommended for VL elimination are similar to those for malaria: (a) early case detection and complete treatment, (b) integrated vector management, (c) effective disease surveillance, (d) social mobilization and behavioural changes, and (e) operational research (World Health Organization, 2005, 2012c; Directorate National Vector Borne Disease Control Programme, no date a).

A range of different vector control interventions have been evaluated to control vectors of leishmaniasis. ITNs have proven useful for the control of *P. argentipes* in some communities(Chowdhury *et al.*, 2017, 2019). However, in India IRS has had the greater impact making it the preferred vector control tool in the Indian elimination campaign (Thakur, 2007; Ostyn *et al.*, 2008; Joshi *et al.*, 2009; Muniaraj, 2014). IRS for VL prevention has been used in India since 2005. To be effective IRS must be applied at a coverage and quality that should achieve the desired impact (Chowdhury *et al.*, 2011; Huda *et al.*, 2011). Resistance in the local vector to the insecticide used for IRS is also a potential threat to the programme success. DDT-based IRS from 2005–2014 failed to reduce transmission levels, in part due to coverage and quality of the IRS and high levels of resistance to DDT in *P. argentipes* (Coleman *et al.*, 2015). In 2015 control efforts switched to a more effective (based on bioassay data analysis) insecticide, alpha-cypermethrin 5% wettable powder, to overcome resistance (Directorate National Vector Borne Disease Control Programme, 2016b) and, stirrup pumps were replaced with compression pumps (Hudson X-pert Sprayer) to improve the quality of IRS delivery and increased training and monitoring efforts were implemented to improve coverage rates.

Data presented here show that there has been an obvious improvement in the IRS programme (based on IRS quality assurance and coverage data) and VL elimination targets (<1:10,000 population) are close to being achieved in the region, prompting WHO to work with Bangladesh, India and Nepal to establish the data requirements for validation of elimination.

Routine entomological and IRS surveillance was embedded in the operational programme in 2016 to determine the impact of the IRS changes in the three VL endemic States; Bihar, Jharkhand and West Bengal (Figure 17).

The WHO target for IRS coverage is >80% of targeted structures being completely sprayed (World Health Organization and TDR, 2010; World Health Organization, 2015). Self-reporting of IRS coverage by the spray teams suggests that >80% of households and >90% of cattle sheds were fully sprayed (Figure18A). Initial independent household survey data suggests that this is an overestimate, with a lower complete spray coverage range (28% to 77%), although 80% is reached if partial house sprays are included. The partial spray can in part be accounted for by the homeowners only allowing certain rooms such as bedrooms to be sprayed while not allowing storerooms or cooking areas to be sprayed. While the expected over reporting by the spray teams continues, there has been improvement in the overall trend towards higher complete spray coverage from 2016 to 2019, this is due to better engagement with communities on spray campaigns and the need to spray the complete structure. The largest improvement was seen in Godda, where actual coverage increased from 28% in 2016 to 75% in 2019.

For an increase in IRS coverage to have the desired impact, the correct dose of insecticide must be deposited onto the surface (Huda *et al.*, 2011; Chowdhury *et al.*, 2014). When DDT was applied using stirrup pumps the quality of IRS was well below that required (Directorate National Vector Borne Disease Control Programme, 2007; van den Berg, 2009; Coleman *et al.*, 2015). Factors reducing the quality of the IRS included a sub-WHO specification formulation or the use of expired insecticide (Directorate National Vector Borne Disease Control Programme, 2007), rapid settling of the formulation in the pumps, compounded by the use of stirrup pumps. Spray operator performance has now been enhanced, with improved training, better quality assured formulations which form more even suspensions and the use of compressions pumps.

Quality assurance of the IRS remains an issue. The WHO recommends bioassays using susceptible insect vectors and/or sprayed filter paper analysis by HPLC. The former method is not possible, as no fully susceptible colony of *P. argentipes* exists, and collecting sufficient numbers of wild caught females to do routine bioassays is not feasible given current low densities of sand flies. Deploying the HPLC analysis of filter papers the average concentration of insecticide applied to surfaces was consistent from 2017 to 2019 (2017: 40.03mg/m^2, 2018: 41.64mg/m^2 and 37.92mg/m^2). This is 1.6 times above the target dose of 25mg/m^2. The reported level of over spraying is likely due to spray operators realising that the filter papers are being checked and ensuring that the papers are well sprayed. The actual amount of insecticide spayed per State is in line with the number of structures calculated to be sprayed at the target dose. An alternative method of measuring alpha-cypermethrin on walls, without the need for filter papers, is now in late-stage development (Kot *et al.*, 2018) and should improve IRS quality assurance further.

Phlebotomus argentipes susceptibility to alpha-cypermethrin and other insecticides that might be used for IRS was monitored following the WHO insecticide resistance testing procedures (World Health Organization, 2016b). As no diagnostic dose for resistance detection has been determined for sand flies, the WHO diagnostic dosages for malaria vectors were used as a surrogate.

The DDT *Anopheles* diagnostic dose works as a good surrogate. DDT resistance has increased from 2016–2019 (66.67% mortality in 2016 to 39.90% in 2019 (Chi^2 $P<0.01$), which is higher than when DDT was used for IRS (Coleman *et al.*, 2015). This suggests that DDT resistance is still being selected for in Indian sand flies. There are two potential sources of selection. DDT is an extremely stable insecticide which decays slowly over many years. It is possible that the sub-lethal doses of DDT remaining on walls in areas sprayed for almost a decade with DDT are still exerting a selection pressure. Alpha-cypermethrin may also select directly for DDT resistance. DDT and pyrethroids (alpha-cypermethrin) both target the *para* voltage-gated sodium channel in the insect nervous system (Rinkevich, Du and Dong, 2013). Mutations in this channel gene, known as *kdr*, (knockdown resistance) are found at high frequencies in *P*. *argentipes* populations in Bihar (Gomes *et al.*, 2017).

While the *kdr* gene strongly predicts DDT resistance in *P*. *argentipes*, our bioassays with alpha-cypermethrin suggest that resistance conferred to this insecticide by *kdr* is likely to be low. IRS with pyrethroids for VL control has been used in Bangladesh with deltamethrin since 2012 (Chowdhury *et al.*, 2014) and Nepal has used alpha-cypermethrin and lambda-cyhalothrin on a rotation since 1992 (Nepal and Epidemiology & Disease Control Division, 2000) with no evidence of resistance selection.

As the diagnostic dosages for Anopheles insecticide resistance testing procedures are likely to be higher than those for sand flies; ideally the diagnostic concentration for monitoring sand flies should be ascertained to improve resistance monitoring and inform insecticide selection. Currently alpha-cypermethrin is an effective insecticide for IRS control of *P*. *argentipes* in India. However, if IRS post-COVID-19 needs to be maintained for several more years, a proactive approach to insecticide resistance management (World Health Organization, 2012b) rotating through different classes of insecticide for IRS should be adopted.

The reduction in peak abundance of *P*. *argentipes* ranged from 81% to 86% per sentinel site over the study period, that exceeds the 67% reduction that models suggest is required for reaching the VL elimination target (Stauch *et al.*, 2014) (Figure 20). The declining trend in numbers of *P*. *argentipes* being caught suggest that the prolonged and extensive vector control programme has had a positive impact, lowering the abundance of vectors and reducing the transmission potential. A decline in *P*. *argentipes* has occurred in both the IRS and non-IRS villages. This may be explained by

the close association of IRS and non-IRS villages in blocks and the potential spill over effect of the IRS control programme. As this was not a cluster randomised trial it is not possible here to compare the two village types directly. However, there is a need to ensure that the trends are not linked to long term sand fly population changes due to climatic variables (Deb *et al.*, 2018).

Across all three states between 2017 and 2019 we detected only 0.03% of *P*. *argentipes* infected with *L*. *donovani* in IRS villages. This is significantly lower than observations in previous studies where *P*. *argentipes* infected with *L*. *donovani* ranged from 0.85% to 32% (Kumar *et al.*, 2001; Tiwary *et al.*, 2012, 2013; Gajapathy *et al.*, 2013; Uranw *et al.*, 2013). This low level of infection detected is due to both the impact of the case detection and treatment reducing the human reservoir of *L*. *donovani* (Singh *et al.*, 2021) and the impact of IRS that will reduce the abundance and age of the vector (Faraj *et al.*, 2013, 2016). The low abundance and low level of infection of *P*. *argentipes* suggests that active transmission in the region is now low, which is evidenced in the reduction in cases (Table 19).

The overall improvement in coverage and quality of IRS is associated with a reduction in the abundance of *P*. *argentipes* and the percentage that are infected with *L*. *donovani*. *C*ombined with improved VL case detection and management the overall impact has been a reduction in disease transmission, which has allowed India to approach the VL elimination target of 1 in 10,000 VL cases at the block level (World Health Organization, 2016a). Annual cases in India have gone from 32,803 in 2005, peaking at around 44,000 in 2007 and then declining by 90% to 3,128 cases in 2019 (World Health Organization, no date a).

The biannual rounds of IRS are timed to coincide with optimum sand fly abundance patterns. From 2005 to 2014 this was often an issue with spraying starting late. Efforts have been increased in recent years to improve the timing of the IRS rounds. However, the second round of IRS in 2018 did not occur, due to operational issues. At this critical stage of approaching VL elimination targets, it is important that entire rounds of IRS are not missed as this could allow for a resurgence of *P*. *argentipes* and increase the selection pressure for insecticide resistance as the residues on the wall from the first-round diminish. An increase in the numbers of VL cases may then be triggered. The COVID-19 pandemic in 2020 delayed IRS activities, however, two rounds were completed in Bihar, Jharkhand and Utter Pradesh, while only one was completed in West Bengal. As India emerges from the COVID-19 pandemic, the impact of this on VL elimination efforts will need to be assessed.

5.5 Conclusion

Due to the improved case detection, treatment and vector control, transmission of *L*. *donovani* by *P*. *argentipes* in India is currently low, which is evidenced in the reduction in cases. This success suggests that it is time for the programme to orientate in line with the WHO VL elimination guidelines(World Health Organization, 2005, 2012c) to the consolidation phase. In this phase the total coverage by spraying may no longer be required. At the same time there is a need for enhanced surveillance to detect increases in VL incidence or changes in the sand fly population so that any potential disease resurgence will elicit a rapid response.

6 Conclusions

6.1 Thesis Conclusions

The benefits of IRS to VL control in India were first noted as a secondary impact to malaria eradication efforts, however the first public health programme targeting VL, which included the IRS strategy, was recorded in 1977 (Deb *et al.*, 2018). Historically efforts to address VL as a public health issue have been reactive to surveys led by groups outside of the Indian national programme, which may potentially indicate that health systems and infrastructure in India were not equipped to systematically monitor disease trends and make rapid informed changes in policy or programmatic efforts. Previous claims of a cyclical trend of VL cases over many years, did not factor in the change in data sources and switch from passive case monitoring to active case detection, however due to the lack of available datasets for fine scale assessment definitive analysis of historical VL trends was not possible (Deb *et al.*, 2018).

Since 2005, the Kala-azar Elimination programme in India has provided free access to VL diagnosis and treatment, with Government funded IRS of structures falling within VL endemic regions. Data published in 2015 was the first multi-site study to collect entomological indicator data for programmatic decision-making (Coleman *et al.*, 2015). This study demonstrated the need for significant changes in the Kala-azar Elimination Programme vector control strategy and changes in insecticide and pump type were introduced.

During the time period of this PhD, the Kala-azar Elimination Programme led monitoring and evaluation activities, largely used IRS coverage survey data as an indicator of performance, which could be coupled with independent spray activity monitoring activities from external stakeholders (e.g. CARE India). Some entomological monitoring studies were conducted by local government research organisations, such as the Rajendra Memorial Research Institute of Medical Sciences, Patna (Kumar *et al.*, 2020), however their dataset was localised to one district in Bihar, making it unsuitable for programme wide decision-making or progression monitoring. This thesis details the system establish in collaboration with the NVBDCP and data collected to monitor the relevant entomological and epidemiological indicators required for informed monitoring of programme progress to date, as per WHO guidelines for effective IRS monitoring (World Health Organization, 2006b). As the Indian programme approaches its VL elimination target, WHO validation to confirm it is no longer a public health issue in India is required. Data requirements for this validation are extensive and include entomological monitoring datasets (World Health Organization, 2016a); datasets collected for this PhD programme will address all required indicators and therefore provide evidence that appropriate entomological monitoring in conjunction with IRS has been conducted. For the purpose of effective data management from multiple sites the DDMS, a decision-support system adapted for the VL and Indian environment, was used (Foster *et al.*, 2017).

Whilst the thesis covers IRS QA within the Indian context only, lack of systematic quantitative measurement of the insecticide delivered onto sprayed structures is a disease agnostic and global issue. The current WHO approved methods for IRS QA are time consuming, require specialist skills and resources and provide results at too late a timepoint to realistically feedback to the spray teams to address performance issues (Russell *et al.*, 2014). Whilst repetitive use of filter papers can make operators aware their performance is being monitored, leading to operational bias. Post-IRS sample collection methods are not replicable (e.g swabs or Sellotape sampling followed by HPLC), or feasible within an operational setting for routine monitoring (e.g. scrapings off walls followed by HPLC) (Russell *et al.*, 2014). The thesis looks at a range of alternative sampling methods, both pre- and post-IRS, to determine if a better surrogate to the WHO filter paper method could be identified. Whilst the thesis demonstrates that other collection methods cannot be directly matched with a filter paper reading, the other collection methods should not be discounted as viable options, as there are also other factors to be considered before adopting the WHO approved filter paper method; most notably, that affixing filter papers onto walls and retaining an appropriate distance between the filter paper and the wall can be problematic. Should the filter paper attach directly to the wall, run down can be a significant issue and the potential for the filter paper to be over dosed with insecticide. Another issue may be that the filter paper does not stay in position or is moved by household members after spraying, potentially making the sample unretrievable. Further exploration of alternative sampling methods for routine IRS QA monitoring is essential in promoting responsible stewardship of insecticides in public health and prolonging the life of currently available active ingredients for IRS.

When considering the post-IRS DQK prototype test kit, designed to detect residual DDT on walls sprayed during IRS, the version tested in the field showed that the kit components had fundamental performance issues. This coupled with the variable sampling efficiency when extracting samples from different surface types, meant that the current prototype was not be suitable for use in a field setting. For wider adoption of alternative strategies, products would need to be robust and withstand poor handling, whilst also providing clear, easily interpretable, and rapid results which can result in action when performance is suboptimal: including refresher training in IRS and respraying of structures. Where surfaces have been over sprayed, this suggests wastage of insecticide, which should also result in retraining. The novel method shown to be effective in detecting insecticide on walls using electromagnetic wave sensor technology (UK- GB1906691.9 and US-16/410,081 patent pending) which we are now working on as a result of our VL activity in India is promising (Kot *et al.*, 2018; Deb *et al.*, 2021) and should be developed further. In summary innovation within the operational vector control landscape has been slow and primarily focused on introducing alternative vector control strategies, with little emphasis on how to effectively monitor current strategies. Improvement in this

area should help ongoing vector borne disease elimination programmes in India and other disease endemic countries.

6.1 Future Research

Whilst this research has provided an extensive overview of the historical VL data available, and contributed new datasets for more sophisticated analysis, there are still some fundamental questions that remain unanswered. These include:

1. What are the spatial and temporal drivers of VL transmission? Are there climatic factors that should be monitored long-term as an early warning of potential resurgence of VL as a public health issue in India?
2. What proportion of the impact seen on VL elimination over the period of this thesis, is attributable to programmatic efforts versus external changes, such as improvement of house quality, reduction of the potential breeding sites for *P.argentipes* sand flies, changes in transport infrastructure, population migration, or improvement of water and sanitation?
3. Are achievements within the Kala-azar elimination programme to date sufficient to break the VL transmission cycle or can we expect a resurgence after certification of elimination of VL as a Public Health problem?
4. What is the impact of prolonged IRS targeting VL on other vectors for diseases of public health importance in the region such as lymphatic filariasis and malaria? When the VL IRS is withdrawn will these increase?

Quality assurance of IRS is essential for performance monitoring and insecticide stewardship from all stakeholders within vector control, however, it is rarely implemented. Therefore, there is a need to ascertain the market drivers and potential hurdles for wide-scale adoption of IRS QA including:

1. Who are the key stakeholders to ensure better adoption and implementation of IRS QA?
2. For novel technology to be adopted and promoted by key influencers, such as WHO, what is the appropriate route for approval?
3. What are the key hurdles preventing programmes from including IRS QA in current practices?

In addition, a longitudinal cost analysis would be advantageous to determine if there are any cost-savings long-term from incorporating IRS QA. This should be coupled with epidemiological data to detect trends in disease transmission.

If point of use post-IRS QA technology does become available as a viable option for performance monitoring, the sampling framework to detect performance at the individual, team and geographical

areas would be advantageous. In addition, there would be an opportunity to couple entomological assay data from cone bioassays with quantitative data on the residual insecticide levels present on walls at the time of the test.

6.2 Study Limitations

The thesis focuses on the hurdles associated with VL elimination in India. It primarily covers the vector control strategies and appropriate entomological monitoring indicators. Climatic indicators are known to be important variables when considering disease and vector trends for malaria (Thomson *et al.*, 2005; Caminade *et al.*, 2014; Nissan, Ukawuba and Thomson, 2021). Whilst this relationship was explored with historical data, this four-year study did not include this component in the analysis. Furthermore, in addition to insecticide susceptibility status and sand fly density, the monitoring of knock-down resistance markers (Gomes *et al.*, 2017) would also be of particular interest, as both DDT and alpha-cypermethrin resistance can be caused by mutations in the *para* voltage-gated sodium channel. There are many unknowns about *P. argentipes* sand fly behaviour, such as breeding behaviours, which could not be incorporated into this four year study to understand trends seen by the operational programme. While the physiological status and sex of the sand flies caught were recorded, parity was not recorded as the vector is very small and dissecting large numbers at the study scale was not considered feasible.

Data sets used to assess suitability of pre- and post- IRS sampling strategies in comparison to the WHO gold standard, Whatman filter papers, was done in an operational setting whereby the spray performance is dependent on the spray operator ability to conduct good quality IRS. Variability in IRS performance in India has been recorded previously (Coleman *et al.*, 2015), and whilst samples were retrieved close to the filter paper, the actual dose delivered in the area could not be verified. This operational issue was also seen with the DDT IQK prototype field study. Both studies were not conducted in controlled settings, e.g. on a wall sprayed with a track sprayer, prior to being taken out to the field and therefore human error leading to spray variability could not be fully considered.

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8 Annexes

Annex 1 – IQK user instructions

ASSURE

Insecticide Quantification Kits (IQK™)

INSTRUCTION LEAFLET (Sampling Tool)
DDT IQK FOR IRS
(Only suitable for)

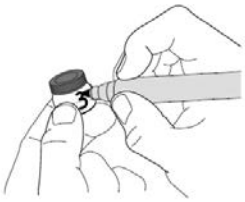
Insecticide Quantification Kits (IQK) for assessing the level of DDT insecticide present on the sprayed surface following Indoor Residual Spray programmes

Test Kit Components
Reagents:
- Reagent A -Red capped bottle (Solution for extraction)
- Reagent B -Green capped bottle (Chloride ion release solution)
- Reagent C – Yellow Capped bottle (Stop Solution)

Equipment:
- 4 Syringes marked as follows:
  Reagent A -Red
  Reagent B -Green
  Reagent C – Yellow
  Blank – Transfer of extraction
- 22 x Screw Top Jars
- 22 x Glass Bottles
- 2 Boxes containing Bottle Caps
- Sample tray for holding marked sample bottles
- Adhesive Circles
- Quantab Strips
- DDT Quantitive Scale
- DDT Record Sheet
- Instruction Leaflet
- Reagents Material Safety Data Sheets
- 2 Sets of Negative Controls
- 2 Sampling Tools

Step 1-Sampling in the field

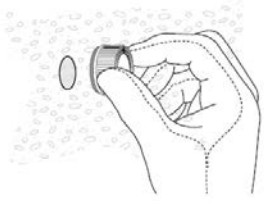
A sample of surface deposit of insecticide is taken from the treated surface using adhesive circles as illustrated below. These samples should be taken from randomised positions



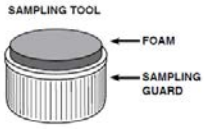
Write the number on jar side and lid corresponding to the sample reference number on the DDT IQK Quantification record sheet (i.e. against insecticide, locality, date and type of surface)

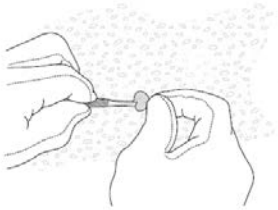
Select a wall to be tested.
Use 2 adhesive circles from the sheet provided.

Place adhesive circles, side by side, onto surface area to be sampled. Ensure good contact by using sampling tool. (Use gloves, do not touch the surface)



Place sampling tool over adhesive circle and press down until the sample guard makes contact with the surface. Repeat the process for the other adhesive circle (Use gloves, do not touch the surface)

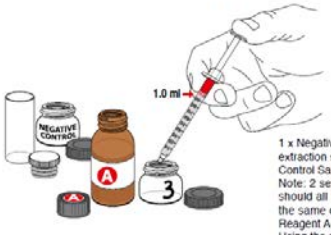




Carefully peel adhesive circles off surface while wearing gloves

Place the adhesive circles into the sample jar

Step 2-Extraction



1 x Negative Control to be included in extraction steps below. Always start with Control Samples.
Note: 2 sets have been provided should all the assays not be done on the same day
Reagent A-Red capped bottle
Using the syringe draw 1.0 ml Reagent A and add to the sample jar containing the sample (marked 3 in above example).

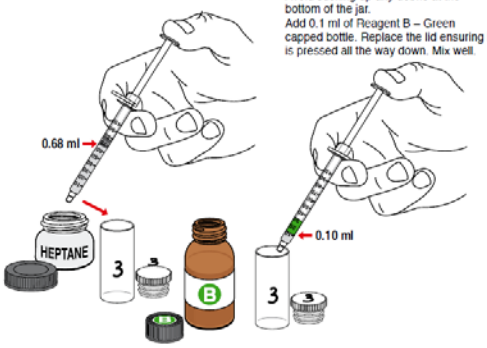
To ensure effective extraction the samples should be positioned flat on the bottom of the sample jar so that samples are fully covered by the solution.

Replace the cap on the jar

Shake well for at least 30 seconds.

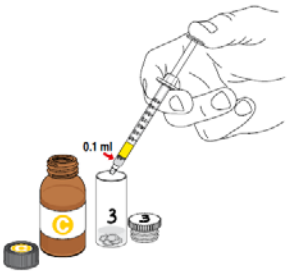
Allow to stand for 2 minutes to allow maximum insecticide extraction from the sample. The sample tray can be used to shake all the jars at the same time

From the top of liquid, transfer 0.68 ml of heptane containing DDT, (using the unmarked syringe), to the glass bottle. Avoid sucking up any debris at the bottom of the jar.
Add 0.1 ml of Reagent B – Green capped bottle. Replace the lid ensuring it is pressed all the way down. Mix well.



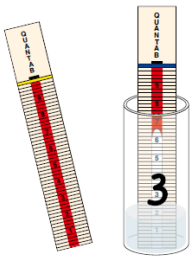
Leave to stand for 60 minutes, shaking periodically every 15 minutes.

Step 3-Quantification



Add 0.1 ml of Reagent C – Yellow capped bottle. Replace the lid ensuring it is pressed all the way down.
MIX VIGOROUSLY.

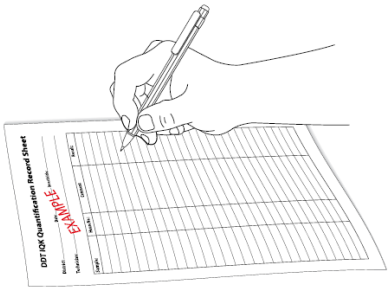
Leave to stand for 1 minute to allow organic (top) and water (bottom) phases to clearly separate.



Place Quantab Strip into the reaction tube, ensuring wick is in the bottom aqueous phase.
The strip should be placed so that the bottom of the strip sits on the flat portion of the bottle base at an angle.
Wait until the Yellow Strip at the top of the dipstick turns Dark Blue, indicating saturation.
Should the yellow stripe turn Red instead of blue, the test should be discarded. This indicates Heptane adsorption.

Quantab	DDT g/m²
1	0.000
1.2	0.027
1.4	0.081
1.6	0.136
1.8	0.193
2	0.252
2.2	0.313
2.4	0.377
2.6	0.443
2.8	0.511
3	0.583
3.2	0.659
3.4	0.738
3.6	0.822
3.8	0.911
4	1.006
4.2	1.109
4.4	1.220
4.6	1.341
4.8	1.477
5	1.631
5.2	1.810
5.4	2.030
5.6	2.314
5.8	2.730
6	3.670
6.2	4.388
6.4	4.790
6.6	5.019
6.8	5.222
7	5.399
7.2	5.538
7.4	5.669
7.6	5.786
7.8	5.894
8	5.994
8.2	6.087
8.4	6.174
8.6	6.257
8.8	6.336
9	6.409
9.2	6.480
9.4	6.548
9.6	6.610
9.8	6.675
10	6.736

Measure the silver chloride peak height (QuanTab values) vs. DDT quantitative scale. Read the result to the closest division on the scale.



Record values on the DDT Quantification Record Chart. See an example herewith.