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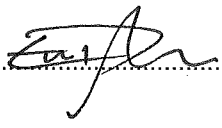
Student's signature:  Date: 16.12.13

The effects of host-vector relationships and density dependence on the epidemiology of visceral leishmaniasis

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Thesis submitted in partial fulfilment of the requirements for the degree Doctor of Philosophy.

University of Warwick,
School of Life Sciences

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Declaration

This thesis is submitted to the University of Warwick in support of my application for the degree of Doctor of Philosophy. It has been composed by myself and has not been submitted in any previous application for any degree.

The work presented (including data generated and data analysis) was carried out by the author except in the cases outlined below:

Chapter 2: All human Montenegro Skin Test (MST) data, canine serology data by immunofluorescence assay test (IFAT) and sandfly count data from Marajó collected between1992-5 were collected and provided by R. J. Quinnell.

Chapter 2: All human Montenegro Skin Test (MST) data, canine serology data by enzyme linked immunosorbent assay (ELISA) from Marajó collected between 2004-5 were collected and provided by O. Courtenay.

Appendix B: The methodology for the collection of human MST and canine serology by IFAT and ELISA as presented in Chapter 2 were provided by O. Courtenay.

Summary

In Latin America, visceral leishmaniasis (VL) is caused by infection with *Leishmania infantum*, an endemic but lethal parasite transmitted by *Lutzomyia longipalpis* sandflies. Multiple hosts are implicated in VL transmission; therefore sandfly biting preferences may be pivotal in determining transmission dynamics. Host preferences are poorly understood with simple preference-host density relationships being conventionally assumed.

Combined modelling and fieldwork approaches were used to investigate the preference of sandflies for key host types (dogs, humans and chickens) and force of infection (FOI) over a range of vector and host densities. In Brazil, variable vector densities were (i) observed over a period of seasonal variation, and (ii) experimentally manipulated via "trapping out" (sustained CDCLT capture to reduce local vector density). Host density was also manipulated by (iii) the incremental introduction of chickens to experimental sheds.

Results suggest that there is a significant link between alternative host density and the absolute and relative preference of sandflies for humans and dogs. Investigations also indicate that host choice has a vector density dependent element, which varies significantly and nonlinearly depending upon vector density. Meta-analysis and mathematical modelling of human and canine prevalences across Brazil also point toward variable transmission rates to these hosts attributable to density-dependent biting preferences observed in the field. These host choice dynamics ultimately combine to demonstrate the influence of host and vector densities on FOI on dogs and humans, but there are significant interactions between host and vector densities resulting in complex FOI relationships.

Nonlinearities are likely explained by density dependent sandfly aggregation behaviour upon outdoor living hosts, such as chickens, as vector density rises. This preference behaviour may have far reaching implications for our understanding of transmission and control, and potentially indicate host density manipulation as an intervention measure.

List of abbreviations

BA	Bacabal
BV	Boa Vista
CDCLT	Centre for Disease Control light trap
CDC_{ON}	CDCLT with light
CDC_{OFF}	CDCLT without light
CDC_{MOD}	Modified CDCLT
CI	Confidence interval
CL	Cutaneous leishmaniasis
DCL	Diffuse cutaneous leishmaniasis
ECD	Effective chicken density
ELISA	Enzyme linked immunosorbent assay
EIR	Entomological inoculation rate
FML	Fucose mannose ligand
FOI	Force of infection (proxy)
GLM	Generalised linear models
GLMM	Generalised linear mixed models
HLC	Human landing catch
ID	Identity
IFAT	Immunofluorescence assay test
IFD	Ideal free distribution
IRS	Indoor residual spraying
ITN	Insecticide treated net
L. infantum	*Leishmania infantum*. Also known as *L. chagasi*, the causative parasite of zoonotic VL
LLIN	Long-lasting insecticide net
LST	Leishmanin Skin Test
Lu. longipalpis	*Lutzomyia longipalpis*. The key vector of zoonotic VL in Latin America.
MCL	Mucocutaneous leishmaniasis
MLE	Maximum likelihood estimation
MoH	Ministry of Health
MST	Montenegro Skin Test
NTD	Neglected Tropical Disease
NS	Non significant
P.	*Phlebotomus* spp. The key genus of leishmaniasis vectors in the Old World.
PCR	Polymerase chair reaction
PKDL	Post kala-azar dermal leishmaniasis
R_0	Basic reproduction number
SE	Standard error

SIR	Susceptible, Infected or Recovered. Deterministic model for disease transmission.
SP	São Paulo
VC	Vectorial capacity
VL	Visceral leishmaniasis
WHO	World Health Organization

Chapter 1: **General introduction and literature review**

1.1. General introduction

Visceral leishmaniasis (VL) is a fatal vector-borne disease thought to infect around 500,000 people annually (Desjeux, 2004), and constitutes a serious public health risk in countries throughout the tropics and subtropics. Yet, over 90% of VL transmission occurs in just five countries, Nepal, India, Bangladesh, Sudan, and Brazil (WHO, 2009), where the burden of disease falls most heavily on the poor and children less than 10 years old (Badaro *et al.*, 1986).

In Latin America the causative agent of VL is the obligate intracellular parasite *Leishmania infantum*. *L. infantum* is principally a zoonotic infection, whereby humans are actually a "dead end" with respect to transmission and the infection is instead maintained within an animal reservoir, namely domestic dogs (Courtenay *et al.*, 2002a). *L. infantum* is transmitted between dogs and to people by the bite of infected female sandflies of the *Lutzomyia longipalpis* species complex (Maingon *et al.*, 2003; Ward, 1983; Ward *et al.*, 1986). Consequently, local vector presence is a prerequisite for transmission to both populations (Caldas *et al.*, 2002), yet there is significant variation in the infection rate between regions. This variation is likely to reflect differences in local vector abundance and host population composition which impact upon infective vector biting rate (Dye *et al.*, 1993). However, overall, the relationship between infection rate in reservoir, accidental hosts (humans) and vector populations remains poorly characterised, with the general assumption being that infection rates in these interacting host populations are linearly related to one another (Dye, 1996).

It is proposed here, however, that differences between regions may also be significantly influenced by heterogeneity and nonlinear density dependence in the vector biting preference for susceptible hosts. Quantifying and understanding heterogeneity in biting preference is of direct relevance to controlling the disease, as the existence of heterogeneities and "hot spots" in transmission present additional opportunities and challenges to control depending on whether interventions can be effectively targeted to areas of high transmission. Heterogeneity is an important determinant of infection dynamics and therefore it is vital to understand dynamics and develop accurate transmission models, in order to make predictions of intervention effectiveness and plan effective leishmaniasis control.

1.2. Literature review

This review is aimed at understanding the epidemiology of leishmaniasis. Epidemiology developed as an independent subject as a study of development of infectious disease, but has since developed principally around non-infectious disease (e.g. cancer, heart disease) in response to the growth of the relative importance of these diseases in the more developed countries during the last century. In terms of understanding the population level patterns of disease, infectious disease is distinguished from non-infectious disease by the fact that an individual's risk of disease is dependent on the status of others in the population. In the case of malaria, the prevalence of individuals infectious (to mosquitoes) determines the prevalence of infectiousness in the vector population, which in turn determines the incidence of infection in people. *Leishmania* in Brazil is different in that the prevalence of human

infection does not drive transmission, which cycles in the dog population. It is the sharing of vectors between dogs and humans that is the subject of this thesis.

1.2.1. Epidemiology of leishmaniasis

The term leishmaniasis refers to a range of diseases caused by parasitic protozoa of the *Leishmania* genus (Kinetoplastida: Trypanosomatidae), which typically multiply within a vertebrate host and are transmitted between hosts and to humans by the bite of infected phlebotomine sandflies.

The leishmaniases are endemic to 98 countries throughout the tropics and sub-tropics, and currently affect over 12 million people worldwide, with an additional, 350 million people are thought to be at risk of infection (WHO, 2010a). The majority of those at risk are from developing countries and among the poorest people in society, where leishmaniasis is associated with poor socio-economic status, malnutrition, illiteracy, population displacement, gender discrimination, poor immune status (Alvar *et al.*, 2006; Desjeux, 2001, 2004) and, increasingly, urbanisation (Desjeux, 2002). Subsequently, due to the individual expense of treatment and interruption to development programmes resulting from outbreaks of disease, the leishmaniases are also associated with the propagation of poverty (Desjeux, 2002).

In acknowledgement of this severe burden which falls unequally on the poor, the World Health Organisation (WHO) have designated the leishmaniases Neglected Tropical Diseases (NTD) (WHO, 2013) and the World Health Assembly approved a Resolution in 2007 (WHA60.13) for the control of leishmaniasis, with the principal

aims to improve awareness of leishmaniasis, improve access to affordable healthcare by promotion of cost reduction amongst drug producing laboratories, standardisation of diagnosis and evaluation of current medicines, to promote of collaboration and development of new interventions and provide expert policy guidelines on transmission prevention (WHO, 2010b).

However, the epidemiology of leishmaniasis is highly complex and throughout the world a number of different parasite and vector species are responsible for infection and transmission (see Appendix A). Different *Leishmania* species or species variants are associated with different vertebrate hosts, leading to different transmission cycles, for example anthroponotic vs. zoonotic, but also different clinical manifestations (see Appendix A). Depending on the etiological agent, disease presentation can vary from ulcerative lesions of the skin or facial mucosa, known as cutaneous leishmaniasis (CL) and muco-cutaneous leishmaniasis (MCL) respectively, to the more serious visceral leishmaniasis (VL), which primarily affects the internal organs, and is usually fatal within 2 years if untreated (WHO, 2010a), but also results in a high mortality rate of 10-20% among treated cases (Desjeux, 2004). Less common clinical disease manifestations also include diffuse cutaneous leishmaniasis (DCL) and post kala-azar dermal leishmaniasis (PKDL), whereby nodular lesions appear all over the body. Though these two may appear similar, DCL is a clinical manifestation associated with CL causing parasite species, whereas PKDL is a cutaneous episode following the resolution of the a VL infection, and is only seen in Asia.

The *Leishmania* parasites that cause this wide range of infection are taxonomically split into two subgenera *Leishmania* and *Viannia*. Members of the *Viannia* subgenus

are restricted to the New World and are associated with cutaneous disease, and in particular the severe presentations such as MCL which are by and large caused by infection with *L. (V.) brazilienses* and *L. (V.) panamaensis.* Parasites of the *Leishmania* subgenus are, by contrast, found throughout the world and are responsible for many instances of CL, but also the deadly visceral disease (see Appendix A for detailed overview).

Of the 2 million new leishmaniasis infections which occur annually, 500,000 are due to VL alone (WHO, 2010a), with 90% of cases occurring in just five countries; India, Nepal, Bangladesh, Sudan and Brazil (Desjeux, 2004). Infection with VL is associated with 50,000 deaths each year, and the loss of 2,357,000 disability adjusted life-years, yet *Leishmania* related mortality is still thought to be underreported (Alvar *et al.*, 2012). Therefore VL is ranked 9th in the global analysis of infectious disease and constitutes a serous health risk.

In the Old World VL is caused by infection with *L. (L.) donovani*, and is transmitted as part of an anthroponotic cycle, whereby the parasite is transmitted between people by the bite of infected sandflies (Desjeux, 2004). In Brazil and the rest of the New World, however, VL is caused by *L. (L.) infantum*, involving an animal reservoir. Here, humans are not reservoirs of disease but "accidental" hosts that represent a "dead end" in terms of parasite transmission (Hotez *et al.*, 2008). People become infected via the bite of infected female *Lutzomyia longipalpis* sandflies which have fed upon zoonotic reservoir hosts, such as the domestic dog.

Many species have been proposed as reservoirs of *L. infantum* in the New World such as the crab eating fox, *Cerdocyon thous*, and the white-eared opossum, *Didelphis albiventris* (Sherlock, 1996; Sherlock *et al.*, 1984) (see Appendix A for full list). However, though potentially of importance to sylvatic transmission, these species make little contribution to peridomestic transmission compared to dogs, *Cannis familiaris* (Courtenay *et al.*, 2002a). The long-lived nature of VL infection in dogs, often in excess of 2 years before the animal succumbs (Hart, 1989), the contribution made to transmission by both symptomatic and asymptomatic animals (Molina *et al.*, 1994) and the readiness with which sandflies feed on dogs (Quinnell *et al.*, 1992) ensure that the domestic dog is the key VL reservoir (Quinnell and Courtenay, 2009).

1.2.2. Sandfly vectors of leishmaniasis

The sandfly vectors responsible for the transmission of the leishmaniases belong to the family Psychodidae, subfamily Phlebotominae. Close to 1000 species of sandfly belong to this subfamily, but only the genera *Phlebotomus, Lutzomyia* and *Sygentomyia* have the bloodfeeding habit. Of these, only sandflies of the *Phlebotomus* and *Lutzomyia* genera have been incriminated as vectors of disease (Service, 2008).

Phlebotomus spp. are restricted to the Old World, where the medically most important species are *Phlebotomus papatasi* and *P. argentipies*, which are the key vectors of CL and VL respectively, across much of the Old World. By contract, *Lutzomyia* spp. are distributed throughout many regions of Central and South

America and occur only within the New World. Here, a key vector in the transmission of CL is *Lutzomyia flaviscutella.* However, it is members of the *Lu. longipalpis* species complex which are most medically important as they are the principal vector of *L. infantum* in the New World.

1.2.3. Lutzomyia longipalpis *(Diptera: Psychodidae) species complex*

Lutzomyia longipalpis sensu lato exists as a species complex (Uribe, 1999), whereby the species is made up of a group of at least three sibling species (Lanzaro *et al.*, 1993; Mukhopadhyay *et al.*, 1998) (Maingon *et al.*, 2003). The term sibling species refers to closely related organisms that are, however, reproductively isolated. Differences in the local vector subspecies are likely to be highly relevant in determining transmission dynamics, as sibling species may exhibit different host preferences, behaviours and susceptibility to *Leishmania* infection and occupy different ecological niches (Mutebi *et al.*, 1999; Rivas *et al.*, 2008; Uribe, 1999), as has been observed among the vectors of the *Anopheles gambiae sensu lato* species complex in malaria transmission (Coluzzi *et al.*, 1979). However, in the case of *Lu. longipalpis* the exact number of sibling species and their relative vectorial capacity and contribution towards transmission of *L. infantum* remains under investigation (Uribe, 1999).

Each sibling species, however, utilises unique mating aggregation pheromones. Three separate sibling species of *Lu. longipalpis* have now been identified through a combination of genetic cross (Lanzaro *et al.*, 1993)and molecular techniques, including isoenzyme studies (Lanzaro *et al.*, 1993; Mukhopadhyay *et al.*, 1997).

Nevertheless, some authors have instead hypothesised that *Lu. longipalpis* exists in highly polymorphic heterogeneous populations, rather than distinct species (Mukhopadhyay *et al.*, 1998; Mutebi *et al.*, 1998). More recent genetic studies using powerful microsatellite tools have suggested that the populations are in fact discrete (Maingon *et al.*, 2003), and identified that the highly dimorphic alleles encoding pheromone genes have gone to fixation in the different populations, indicative of reproductive isolation, although molecular studies may be compromised by the limited ability to detect recent bottlenecks (Maingon *et al.*, 2003).

Of significance to control is that the sibling species employ markedly different communication patterns in mate and species identification and lek aggregation, with males utilising consistently different courtship songs (de Souza *et al.*, 2002) and sex pheromones to attract females to copulate. Such isolation mechanisms are also strongly correlated with phenotypic differences in certain areas such as Sobral, Ceará Sate, Brazil, where flies with either two or one pale tergal spots are characteristic of different subpopulations (Ward, 1983). Three distinct pheromones, 3-methyl-alpha-himachalene, 9-methylgermacrene-B (16-carbon chain molecules (C16)) (Hamilton *et al.*, 1996a;b) and cembreme (C20-molecule) have been identified as indicative of different sibling species (Hamilton *et al.*, 1996c) and are responsible for species-specific attraction of conspecifics into leks. Once attracted to leks, further courtship behaviour involves courtship songs, which differ between burst and pulse type songs between the distinct sibling species (Lins *et al.*, 2012). Contact pheromones and cuticular hydrocarbons also differ between sibling species, and along with song may also contribute to the accurate identification of conspecifics before copulation (Bray and Hamilton, 2007a). A number of these cues may also communicate honest

reproductive signals, such as male fitness, and thus influence intraspecific mate choice (Jones *et al.*, 1998), in addition aiding species identification.

In some areas of Brazil, sympatric populations of sibling species can be found such as Sobral, Ceará Sate, Brazil, however, in many other locations a single sibling species may exist in isolation, such as on Marajó where only the cembreme diterpene producing species resides (Maingon *et al.*, 2003).

1.2.4. Lutzomyia longipalpis *ecology and life cycle*

Lutzomyia longipalpis inhabit many ecotopes throughout Latin America, stretching between Mexico and Argentina (Uribe, 1999). These sandflies are traditionally associated with crepuscular biting activity (Courtenay *et al.*, 2007) and sylvatic disease transmission (Campbell-Lendrum *et al.*, 2001), and therefore transmission to rural human populations. However, 70% of South Americans now live in urban areas, and global trends in urbanisation are associated with changing epidemiology of zoonotic VL (Desjeux, 2004). The increasingly urban habit of sandfly species, as a result of urban encroachment upon the natural habitats of vectors and reservoirs, has led to increased contact between vectors and people which contributes to the emergence of VL as a disease of urban areas (Desjeux, 2004). As such, *Lu. longipalpis* is described as a peridomestic vector due to its colonization of the advancing urban fringe (Service, 2008).

Female sandflies are anautogenous, requiring a bloodmeal to support oogenesis (Soares and Turco, 2003), which they can undergo every 3 days (Dye, 1996). Female

sandflies are usually batch feeders and may lay up to 70 eggs per bloodmeal, although fecundity may vary according to the blood source (Ready, 1979; Service, 2008). Despite extensive research, the location of oviposition in *Lu. longipalpis* remains unknown (Hanson, 1961). Deane and Deane (1957) identified a total of 19 immature *Lu. longipalpis* stages among the soil of animal corrals and rocks in the peridomestic environment, highlighting a large discrepancy between adults and immature fly densities and understanding of their distributions.

Sandflies may complete their life cycle in 32 days, depending upon environmental conditions, such as temperature, and the quality of blood source supporting initial development (Noguera *et al.*, 2006; Ready, 1979; Soares and Turco, 2003). Within 6-9 days of oviposition larvae hatch, and over the next 25 days develop through four larval instars, before pupating for 8-9 days and emerging as adults (Service, 2008; Soares and Turco, 2003).

1.2.5. Behavioural ecology of the sandfly vector

Lutzomyia longipalpis are eclectic in their feeding preference and readily feed upon a wide variety of hosts within the peridomestic environment (Deane and Deane, 1962; Morrison *et al.*, 1993b). Host preference appears to occur as innate function of host biomass (Quinnell *et al.*, 1992), most likely because larger animals produce more kairomone, a key odour attractant of sandflies in the lab (Morton and Ward, 1989). Local abundance and distribution of female flies is also strongly influenced by males (Campbell-Lendrum *et al.*, 1999a) which form lek-like aggregations in association with hosts (Jones and Quinnell, 2002), and produce sex pheromone which works in

synergy with host odour to attract females over distance (Bray and Hamilton, 2007b; Morton and Ward, 1989; Quinnell and Dye, 1994b). Given their relative contribution to household biomass, humans may be expected to be fed upon relatively frequently compared to 1-2 dogs or a small number of chickens per household, but biting preferences are also likely to be determined by host accessibility (Quinnell and Dye, 1994b) and host defensiveness. Therefore, by virtue of being awake during crepuscular hours, which is the peak time for sandfly activity, and being resident within houses, sandfly feeding opportunities upon people are actually limited in comparison to other domestic hosts (Costa *et al.*, 2005; Courtenay *et al.*, 2007; Quinnell and Dye, 1994a;b).

Leks form on suitable vertebrate hosts and provide a location where sandflies aggregate to concentrate their reproductive activity. Lek formation commences in response to potent combinations of host kairomones, from hosts such as chickens, and species-specific male sex pheromone (Lane *et al.*, 1985). The attractive nature of these volatile compounds recruits males to join the lek site, which in turn promotes pheromone production and increased recruitment (Kelly and Dye, 1997). Females, which commence nightly activity later than males, are subsequently recruited to the lek site as a function of the number of male sandflies, specifically entering the lek to have their eggs fertilized and obtain a blood meal from the host to support oogenesis before leaving the lek (Kelly and Dye, 1997). This differential timing and duration of lek-based activity between the sexes gives rise to a male bias in the sex ratio of sandflies often observed within leks (Kelly and Dye, 1997). Due to the attractiveness of leks as a function of pheromone production, larger leks are expected to be more attractive. This lek size dependant response among female flies is potentially due to

the benefits of increased mate choice, sperm competition and increased chance of successful copulation within larger leks (Jones *et al.*, 1998). However, response to leks based on size may simply reflect the fact that larger plumes of sex pheromone are likely to be more noticeable and easier for other sandflies to locate in a heterogeneous environment, especially over distance (Bray *et al.*, 2010).

Host density has been shown to be a significant determinant of fly distribution and density (Fernandez *et al.*, 2010), with multiple studies having identified specific host species, in particular chickens, dogs and number of people per household as significant risk factors for human (Badaro *et al.*, 1986; Dye and Williams, 1993; Fernandez *et al.*, 2010; Quinnell and Dye, 1994a) and dog infection (Miles *et al.*, 1999; Moreno *et al.*, 2005). Chickens are resistant to *Leishmania* infection (Otranto *et al.*, 2010) and therefore are considered only as a sandfly population maintenance host (Alexander *et al.*, 2002). It is the number of infected host animals that are most important to transmission, a relationship highlighted by Oliveira *et al.* (2001) who noted significant linear relationship and spatial-temporal association between seropositive dogs and clinical human cases. However, clinical burden gives a poor indication of human exposure and VL infection prevalence as the ratio of asymptomatic to symptomatic individuals is highly variable, and can vary from 4:1 to 30:1 (Michalsky *et al.*, 2009), plus, clinical cases are typically very low in frequency, and potentially suffer from underreporting (Alvar *et al.*, 2012). Therefore associations between host density and clinical cases should be viewed with caution. Furthermore, the above associations between host density and infection risk have not been consistently reported (Caldas *et al.*, 2002; Fernandez *et al.*, 2010; Moreno *et al.*, 2005), with Moreno *et al.* (2005) failing to identify clinically sick dogs as a risk

factor for infection and Quinnell and Dye (1994a) demonstrating that in fact the relationship between host density and infection risk is more complicated and the presence of additional predictors, such as chicken sheds, can confound the relationship.

1.3. Interventions

In an attempt to control VL a number of different interventions have been devised. These include, dog culling, canine treatments and canine vaccination to reduce the size of infectious reservoir populations, insecticidal applications to reduce vector survival and population size, drug treatments to limit disease burden in dogs and humans and the use of insecticide-treated nets (ITN) and dog collars to provide personal protection. These methods have experienced limited small-scale success in terms of disease control in some areas, but an under-appreciation of vector behaviour and transmission dynamics can potentially explain intervention failure in others.

1.3.1. Reservoir reduction

As dogs are the key peridomestic reservoir of *L. infantum*, the identification and destruction of infected dogs has formed the cornerstone of VL control in the New World for many years (Tesh, 1995).

This controversial mode of reservoir control has been associated with a reduction in the incidence of canine leishmaniasis in China, contributing to the success of large scale VL elimination programmes (Hart, 1989; Leng, 1982). However, the strategies

employed in China relied upon reduction of the whole reservoir population, rather than the selective removal of infected animals. By contrast, in Brazil, only dogs identified as positive immunofluorescence assay test (IFAT) are removed. Here, rapid detection and removal of dogs (within seven days) has demonstrated efficiency, resulting in a 27% (Braga *et al.*, 1998) reduction in seroprevalence 10 months after the intervention when compared to the much slower municipal removal in neighbouring areas. Rapid removal has also been associated with significant reduction in infection incidence from 36% to 6% in intervention areas (Ashford *et al.*, 1998). However, these reductions were also not achieved through culling alone, but culling in conjunction with insecticidal and chemotherapeutic controls.

Despite such some small-scale success, years of government-coordinated culling activity in Brazil have failed to control VL. Between 1990 to 1994 alone, 4.5 million dogs were screened and 80,000 seropositive animals destroyed, but this failed to reduce significantly canine infection rates (Dietze *et al.*, 1997).

The failure of reservoir reductions in the New World is largely a result of serological test insensitivity and long lag times between exposure, infection detection and destruction, which often exceed the average latent period, leading to significant transmission and dilution of intervention efficacy (Courtenay *et al.*, 2002b; Palatnik-De-Sousa *et al.*, 2004; Palatnik-de-Sousa *et al.*, 2001). The intervention is also highly unpopular, therefore the survival of dogs in households which refuse or delay Ministry of Health (MoH) testing, plus the presence of stray animals or sylvatic reservoir hosts in some foci may help to maintain transmission (Dietze *et al.*, 1997). Euthanized dogs are also rapidly replaced, often with susceptible animals such as

puppies and naive immigrant dogs, but also with infected animals brought in from other areas (Nunes *et al.*, 2008). The reintroduction of infected hosts and changes in the canine population structure through the increased availability of susceptible non-native dogs could even promote epidemic transmission rather than aid control (Nunes *et al.*, 2008).

It has been suggested that culling may be more effective in areas of low endemicity, because the loss of each infected dog has a more substantial impact upon transmission potential (Reithinger *et al.*, 2004).

1.3.2. Chemotherapy for dogs

Alternative possibilities for the reduction of the infected reservoir are canine chemotherapeutic treatments. However, despite a variety of treatments being available (Baneth and Shaw, 2002), the current arsenal of antileishmanial agents are not associated with parasite clearance in dogs (Molina *et al.*, 1994).

For example, first line treatment for canine leishmaniasis is with antimonial therapy, a treatment which has been in use for close to 100 years in the control of VL of both humans and dogs (Gradoni *et al.*, 1988). Antimonial treatments are associated with temporary relief from symptoms in dogs and even reductions in the number of clinical episodes (Gradoni *et al.*, 1988) and parasite loads in the skin (Alvar *et al.*, 1994). However, the therapy fails to eliminate parasites in 79% of canines (Deplazes *et al.*, 1992), leading to high relapse rates of over 70% within a year (Koutinas *et al.*, 1999; Moreno *et al.*, 1999), and failure to interrupt transmission (Slappendel and

Teske, 1997). Treatment efficiency may be compromised by high toxicity of antileishmanial drugs which is dose limiting (Koutinas *et al.*, 1999).

Alternatively, slower paced treatment regimens can reduce renal toxicity (Malik *et al.*, 1996) and continuous use can also contribute to long-term clinical control, even helping to prolong life in 78% of dogs infected with *L. infantum* in European foci (Koutinas *et al.*, 1999).

Increased canine longevity without parasitological cure may increase the contribution an infected animal makes to disease transmission over its lifetime. Such outcome would make canine treatment not only counterproductive, but also unethical. Additionally, the prolonged exposure of *Leishmania* parasites to sub-lethal drug doses is likely to select for resistance (Gramiccia *et al.*, 1992), an issue of particular concern given that human treatment relies upon the same suite of chemicals, such that in some countries including Brazil, the use of human drugs for canine treatment is legally prohibited. Finally, the cost associated with long-term treatments or less toxic formulations, such as liposomalized amphotericin B, make them unsuitable for the treatment of dogs in developing countries and unsuitable for large-scale control.

1.3.3. Chemotherapy for humans

Visceral leishmaniasis infection in humans is also treated with the same suit of chemotherapeutic agents as canine VL, with pentavalent antimonials being frontline drugs, except in areas of high antimonial resistance where amphotericin B formulations are the replacements (Ahmed *et al.*, 2012). In contrast to canine

treatment, these drugs are far more effective in people and often result in parasitological cure.

Treatment is, however, compromised by dose-limiting toxicity of existing drugs, high treatment cost, and limited access and treatment options. In particular, there is a lack of new drugs available for treatment (Olliaro *et al.*, 2002; Trouiller *et al.*, 2002), while existing treatments involve lengthy treatment regimens, often requiring periods of hospitalisation, and are suffering from a rise in drug resistance (Sundar *et al.*, 1999) and problems of toxicity and relapse in HIV co-infected individuals (Guerin *et al.*, 2002; Laguna *et al.*, 1999). The economic impact, both in terms of treatment cost and days at work lost, are often prohibitively high to people in developing countries where they may not be able to afford the necessary treatment (Guerin *et al.*, 2002). These considerations highlight the necessity to develop new chemotherapeutic interventions for leishmaniasis at an affordable price (Trouiller *et al.*, 2002) and design and improve intervention tools.

However, human treatment against zoonotic VL does not impact upon transmission because humans are not infective hosts. Therefore, while human treatment remains a necessary tool in the reduction of disease burden, other interventions must be explored if zoonotic VL is to be eliminated.

1.3.4. Canine vaccination

Given the poor efficacy of chemotherapeutics in dogs, there is a pressing need for the development of prophylactic measures. Spontaneous recovery from canine

leishmaniasis is rare but appears to confer immunity in dogs in some cases (Kedzierski *et al.*, 2006), plus there is probable cross immunity between *Leishmania* species (Coler and Reed, 2005), making vaccination a very attractive tool for leishmaniasis control.

In general, vaccine efficacy and uptake are dependent upon vaccines providing long-term protective immunity, being safe, easy to store and administer, and being affordable. Several approaches have been employed to develop such a vaccine for the canine VL reservoir; ranging from live or killed whole parasites, partial parasite fractions, synthetic immunogenic protein or DNA-based vaccines. However, despite these multiple approaches only one prophylactic vaccine, Leishmune (Fort Dodge Animal Health), is commercially available and is licensed only within Brazil. A second vaccine, Leish-Tec (Hertape Calier Saúde Animal), is now also approaching commercialisation (Evans and Kedzierski, 2012). However neither vaccine is recommended by the Brazilian MoH for the vaccination of dogs due to the absence of specified data.

Leishmune, derived from the fucose mannose ligand (FML) purified parasite fraction of *L. donovani* has proved highly effective within murine and canine models (Palatnik-de-Sousa *et al.*, 1994), and in the field. When administered with a saponin adjuvant it confers 95% protection against symptomatic disease among vaccinated dogs, resulting in 80% fewer VL cases among vaccinated dogs compared to controls (Borja-Cabrera *et al.*, 2010). Vitally, this effect is also transmission blocking (Nogueira *et al.*, 2005) and effective in the long-term, with 97% of vaccinated dogs showing no signs of infection within 4 years (Palatnik-De-Sousa *et al.*, 2009). Therefore it makes a two-fold contribution to disease control by protecting dogs from

infection and also preventing them from becoming infectious (Nogueira *et al.*, 2005), making a significant contribution to effective reservoir reduction. Correspondingly, the use of Leishmune in Brazil has been associated with significant reductions of 61% and 25% in human cases and canine seroconversion respectively in the year following vaccination uptake in Araçatuba, Brazil, where only 5.7% of dogs were vaccinated (Palatnik-De-Sousa *et al.*, 2009). However, the national culling and vector reduction programmes were on-going throughout the survey period and these studies lacked the appropriate controls and replicates; therefore, it is difficult to elucidate the effect attributable to the vaccine (Quinnell and Courtenay, 2009). Additionally, only 1.3% of dogs seroconverted in response to vaccination with Leishmune, hence use of Leishmune is unlikely to confound the detection of infection in dogs and lead to the culling of protected dogs if culling and vaccination are used as an integrated means of reservoir reduction (Palatnik-De-Sousa *et al.*, 2009).

It follows that the greatest challenge to control of *Leishmania* through vaccination is uptake. To be most effective, coverage would need to reach a level to induce herd immunity, whereby the disease reservoir and transmission potential is so low that even non-vaccinated individuals are protected from infection. The threshold at which herd immunity is established amongst canines for this vaccine is unknown. However, despite demonstrated efficacy, Leishmune is not a recommended control option in Brazil, and therefore coverage remains low (Hart, 1989). Adjuvant-related side effects, including localised pain and swelling at the inoculation site and temporary anorexia (Parra *et al.*, 2007; Santos *et al.*, 2007), are also potential barriers to uptake

in Brazil as well as barriers to licensing in other canine VL endemic regions, such as Europe.

Second generation vaccines under development include those based on excreted immunogenic compounds of *L. infantum* parasites (Bourdoiseau *et al.*, 2009; Lemesre *et al.*, 2007; Lemesre *et al.*, 2005), recombinant proteins (Carcelen *et al.*, 2009; Molano *et al.*, 2003; Poot *et al.*, 2009) and DNA vaccines (Carson *et al.*, 2009; Gurunathan *et al.*, 1998).

1.3.5. Human vaccination

The availability of a prophylactic vaccine for humans would represent a major step forward in reducing disease burden and reduce the heavy reliance upon costly and toxic drugs. The relative simplicity of the *Leishmania* life cycle, resistance to re-infection in the case of CL and the activation of cell-mediated resistance in experimental models makes it one of the parasitic infections most attractive to vaccine control (Davies *et al.*, 2003; Kedzierski *et al.*, 2006). Nevertheless, the immunology of *Leishmania* infection is complex, and control of human infection via vaccination remains elusive.

For over one hundred years human vaccination against CL has been achieved via inoculation with viable cultured parasites, otherwise known as leishmanisation (Wenyon, 1911). The controlled infection, once healed, confers protection, which has resulted in a reduction of CL cases of over 90% among Iranian army personnel (Greenblatt, 1988). Though unsuitable for VL and MCL control, for which infections are not self-healing, leishmanisation was once widely adopted in regions of the

former Soviet Union, Israel and Iran for control of *L. major* (Nadim *et al.*, 1983). Leishmanisation has suffered due to questions of safety and ethics as the use of unstandardized inoculations of viable parasites, especially in HIV infected people, could contribute to severe infection outcomes and possible epidemics rather than control. Nevertheless, leishmanisation is now undergoing a revival in the control of CL, as genetically stable and standardised inoculums of parasites have become available (Davies *et al.*, 2003).

Safer killed parasite preparations, such as those using *L. amazonensis*, have not shown better efficacy against CL in Colombia (Velez *et al.*, 2000), Ecuador (Armijos *et al.*, 2004) or Brazil (Mayrink *et al.*, 1979), demonstrating only partial protective immunity (50%) against *Leishmania* infection (Greenblatt, 1988). More promising recombinants for the control of VL and CL include Leish 111-f, which, despite failure to prevent infection in canines (Gradoni *et al.*, 2005) is undergoing testing in humans where it is hoped it will confer cross reactivity between species (Kedzierski *et al.*, 2006).

1.3.6. Vector control

Given the dual role of vectors as transmitters between dogs and from dogs to humans, reduction of the vector population (and hence the host to vector ratio and biting rate), as supported by modelling, could prove to be a highly effective means of reducing transmission (Dye, 1996). Reduction in vector density would be expected to have a linear impact upon transmission, but intervention which also effect parameters such

as in vector life expectancy may exert additional nonlinear reduction in transmission too.

1.3.7. Indoor residual spraying

Traditionally, control of leishmaniasis vectors has been attempted via insecticide application to indoor resting and lekking sites of houses and animal sheds, also known as indoor residual spraying (IRS). This insecticidal application is a versatile approach that can be applied to different locations depending on vector host preference, such as on the walls of houses for control of anthropophilic vectors or animal houses for zoophilic vectors.

Anecdotally, intensive household IRS programmes carried out during the 1950s and 1960s in an effort to eradicate malaria were also associated with reductions in the occurrence of anthroponotic VL in South East Asia. However, VL has recurred since the cessation of IRS activity, highlighting that insecticidal effect is lost over time and that sandfly numbers readily recover (Bora, 1999). Additionally, reducing the period over which vectors may be exposed to sub-lethal doses of insecticide will help prevent the rise of insecticide resistance (Ostyn *et al.*, 2008).

Lutzomyia longipalpis, tends to be opportunistic in its feeding preference (Morrison *et al.*, 1993b), but with a tendency to aggregate in animal shelters (Quinnell and Dye, 1994a). IRS activity in Brazil has therefore been focussed on animal shelters. However, IRS appears to have had little impact on VL transmission (Albano Amora *et al.*, 2009) with negligible impact upon vector abundance in some areas (Albano

Amora *et al.*, 2009; Dinesh *et al.*, 2008), though few robustly designed studies are available (Quinnell and Courtenay, 2009). Such failure against some sandfly species may in part be associated with heterogeneous spraying coverage of shelters, which allows the survival and continued aggregation of sandflies in untreated shelters (Kelly and Dye, 1997). This highlights the importance of vector behaviour and spatial heterogeneity in determining control success.

1.3.8. Insecticide treated bednets

Insecticide Treated Nets (ITN) have proven to be highly successful in the control of several vector-borne diseases, including lymphatic filariasis, Chagas disease and malaria (Bogh *et al.*, 1998; Kroeger *et al.*, 1999; Lindsay *et al.*, 1993). ITN are efficacious as they act as a mechanical barrier to biting (Clarke *et al.*, 2001), but also repel and kill vectors that come into contact with them, which ensures continued efficacy even when nets are damaged (Carnevale *et al.*, 1992). Long-lasting insecticide treated nets (LLIN) are now also contributing to the efficacy of bednets over time with longer lasting formulations that do not require regular net retreatment (Guillet *et al.*, 2001). Nets have contributed toward disruption of malaria transmission between humans and mosquitoes, with reductions in sporozoite rates also conferring "mass protection" in some communities (Gimnig *et al.*, 2003; Hawley *et al.*, 2003). In the case of zoonotic VL, however, ITNs will be unable to interrupt the natural transmission cycle and will therefore only constitute a means of personal protection. Yet, even this relatively limited success is reliant upon the degree of vector anthropophily and endophagy and timing of biting activity relative to bedtimes.

In Iran, Sudan and Afghanistan the effect of personal protection has been shown to be significant, and translate into a reduction in the incidence of CL cases due to anthroponotic *L. tropica* (Alten *et al.*, 2003; Jalouk *et al.*, 2007; Moosa-Kazemi *et al.*, 2007; Reyburn *et al.*, 2000; Ritmeijer *et al.*, 2007; Yaghoobi-Ershadi *et al.*, 2006). A cluster randomised control trial of ITN in an area of anthroponotic VL transmission found significant reductions in the number of flies caught indoors associated with ITN use (Picado *et al.*, 2010a), which conferred partial protection from exposure to sandfly bites (Gidwani *et al.*, 2011), likely reflecting the high degree of anthropophily of Old World VL vectors. However, such benefits have not been consistently found (Dinesh *et al.*, 2008), and have not translated into reductions in human infection incidence during trials in India and Nepal (Picado *et al.*, 2010b).

By contrast, New World vectors of VL are not highly anthropophilic (Quinnell *et al.*, 1992), or endophagic (Courtenay *et al.*, 2007), preferring to aggregate outdoors in the early evening. Nevertheless, Courtenay *et al.* (2007) demonstrated that insecticide treatment enhances the barrier effect of wide mesh bednets by 39.2% and increased daily sandfly mortality following prolonged contact with the net (i.e. pass through it) by 97.7%, and in addition reduced sandfly landing rates both under and external to the ITN (Courtenay *et al.*, 2007; Jalouk *et al.*, 2007). These effects did not result in a reduction in sandfly density or entry within ITN using houses relative to controls, and no repellent activity was observed. However, the small sample size of 4 houses sampled over just 15 days, limits the broader application of the study of Courtenay *et al.* (2007).

Nevertheless, reductions in landing rates on people, without changes in vector density, raise the important question of where do sandflies feed. Vector diversion to unprotected hosts could compromise community wide control (Killeen *et al.*, 2002) and ITN usage may also select for zoophagy among vectors (Courtenay *et al.*, 2002b). This may in fact increase VL transmission if it results in increased feeding upon dogs, which could potentially negate any benefits of reduction to human biting.

1.3.9. Topical insecticide applications

A novel method of insecticide delivery to dogs is via an insecticide impregnated collar, bath or pour-on formulation. The logic behind topical applications is not simply that they may protect dogs but more importantly will reduce the life expectancy of flies feeding upon potentially infected reservoir hosts. Scalibor (Merck Animal Health) collars incorporating deltamethrin are efficacious because the lipophilic nature of the synthetic pyrethroid formulation allows the insecticide to be released slowly and subsequently distributed over the animal's skin and absorbed into the dermis (Killick-Kendrick *et al.*, 1997; Miller and Salago, 1985). This ensures that a toxic dose of insecticide is delivered to sandflies over 5-6 months, theoretically irrespective of bite site, showing no long term toxicity to dogs, though it may take up to two weeks for the insecticide to become dispersed throughout the dogs skin (Killick-Kendrick *et al.*, 1997).

On the whole, laboratory experiments have identified that topical formulations show more persistent anti-feeding effect than sandfly mortality effects (David *et al.*, 2001). Insecticide impregnated collars induce strong anti-feeding effects in *P. papatasi*

(Halbig *et al.*, 2000), *P. perniciosus* (Killick-Kendrick *et al.*, 1997; Maroli *et al.*, 2001) and *Lu. longipalpis* (David *et al.*, 2001). However, the use of wild caught flies (Halbig *et al.*, 2000), which may not require a bloodmeal, and small sample sizes may have biased these results.

Lu. longipalpis appear particularly susceptible to this form of control as exposure of flies to collared dogs within netted cages, demonstrated the cessation of feeding on treated dogs for the first 12 weeks post application (David *et al.*, 2001). Feeding activity recovers to only 6% of flies after 9 months of collar use, a dramatic effect compared to 75% engorgement upon untreated dogs (David *et al.*, 2001). Mortality among *Lu. longipalpis* exposed to treated dogs was also significant (96% at 4 weeks post collaring), but steadily dropped (35% by week 35) (David *et al.*, 2001).

It was anticipated that such repellent anti-feeding effects would be beneficial in reducing the rate of canine blood feeding and therefore fly infection rates (Killick-Kendrick *et al.*, 1997). This hypothesis is reliant on the assumption that collar coverage is high and that repellent behaviour does not simply divert flies and increase transmission among the unprotected hosts, such as un-collared dogs, sylvatic reservoirs or humans. As with all insecticidal applications, insecticide-induced changes for alternative feeding hosts and locations (e.g. host switching), and insecticide resistance, are potential barriers to sustained efficacy.

A small scale randomised controlled trial of Scalibor collars in Brazil revealed a non-statistically significant difference in *Leishmania* exposure between un-collared and collared dogs, as measured by ELISA and PCR prevalence, of 17.6% and 11.9%

(*P=0.24*), but a significant 50% (*P=0.01*) reduction in seroconversion risk among collared compared to un-collared dogs (Reithinger *et al.*, 2004). Similarly, another study in Iran demonstrated a significant difference in canine infection rates between collared and control clusters, which also corresponded to a halving in the Leishmanin Skin Test (LST) reactivity rate among children (Gavgani *et al.*, 2002).

Therefore, it appears that insecticide impregnated collars do confer a protective benefit to dogs and are likely to be a highly acceptable control measure for dog owners especially in areas of seasonal transmission, where a single annual application could confer protection (Killick-Kendrick *et al.*, 1997). Maintaining high levels of coverage may however be difficult given the variable rates of collar loss which have been reported to be as high as 41% over a 5-month period (Reithinger *et al.*, 2002). Besides, the effect of manipulating sandfly feeding behaviour in terms of transmission to humans remains unknown.

1.3.10. Pheromone-baited insecticidal traps

Members of the *Lu. longipalpis* species complex produce pheromone cues to coordinate lekking aggregations and mating behaviour (Jones and Hamilton, 1998). This behaviour offers an unprecedented opportunity for targeted control, whereby the natural sandfly aggregation cues can be exploited and used to bait insecticidal traps (Hamilton *et al.*, 1996a;b; Witzgall *et al.*, 2008). Pheromone-derived control has become commonplace in the management of economically important agricultural pests such as the leopard moth (Hegazi *et al.*, 2009) and codling moth (Witzgall *et al.*, 2008), where synthetic pheromone is used in baited traps or disrupt moth mating

activity which is reliant upon pheromone cues. Due to the strong selection pressure for sensitivity to conspecific factors, pheromone disruption has become particularly useful in the face of insecticide resistance. However, pheromone-based controls have yet to be widely investigated in vector control.

Female response to pheromone increases with pheromone production (number of males) (Jones and Quinnell, 2002) and in combination with host kairomones (Bray and Hamilton, 2007b). Therefore, pheromone-baited traps, placed in association with hosts aim at eliciting this attractive response by mimicking the natural odour plume normally associated with lekking behaviour. Baited insecticidal traps are anticipated to kill flies, but also benefit from continued recruitment to the trap site, as the death of males will not impact on pheromone production (Kelly *et al.*, 1996, 1997). This would overcome problems associated with male insecticide-induced mortality, heterogeneity of insecticide application (Kelly *et al.*, 1997) and possible repellent effect of insecticide.

Thus far, synthetic volatiles have only been developed for one of the *Lu. longipalpis* sibling species, which has a distribution across the Americas, from Mexico to southern Brazil (Ward, 1983) and is responsive to 9-methylgermacrene-B (Hamilton *et al.*, 1996a;b). Small scale tests demonstrate a strong attractive and mortality effect of traps baited with this pheromone in the field, with pheromone use resulting in as much as a 20-fold increases in the number of dead flies of both sexes in pheromone-treated sheds compared to controls (Bray *et al.*, 2010). This mass mortality has the potential of reducing both the biting nuisance and VL risk by reducing female sandfly abundance but also via a reduction in the number of males available for the formation of new lekking sites and mating activity (Bray *et al.*, 2010), and possible

manipulation of sandfly distributions actively diverting flies from human and canine hosts. However due to close proximity between test and control huts (3m) and small experimental area (10 sheds over meters) it is not possible to assess any community-wide benefits of this control measure. Large-scale field trials are now under way (Courtenay, pers. comm.).

Should pheromone traps be effective on a wide scale, they hold much promise for effective control, facilitating focal insecticide application (Bray *et al.*, 2009), limiting environmental impacts and selection for resistance in other insect species.

1.3.11. Larval control

As indicated above, insecticide is typically directed toward the adult vector stages and there is little control effort focussed upon preventing adult emergence and development. This is likely due to the uncertainty surrounding the larval habitat of sandfly species (Feliciangeli, 2004), and the difficulty associated with safely targeting insecticide to sandfly larvae over a widespread area. Additionally, sandflies exhibit high mortality between the life-stages (R strategists), with relatively few immature stages developing to adulthood, and even fewer individuals living long enough to contract and transmit the parasitic infection. This makes larval control in general an inefficient intervention (Killeen *et al.*, 2002; Majambere *et al.*, 2010), and it has not been attempted in sandflies.

1.3.12. Environmental vector management

Environmental vector management (EVM) is an alternative approach to vector control without the use of insecticide. In areas of anthroponotic transmission, the local vector, *P. argentipes*, has been associated with breeding sites within the home. To control indoor breeding, mud and lime have been used to fill in cracks, effectively reducing sandfly density (Joshi *et al.*, 2009). However, frequent reapplication is required to maintain such a control strategy, which may be beyond the means of the householder and there is little evidence to indicate that this reduces biting rate within the house.

As the identification and control of vector breeding sites pose a significant challenge, alternatives such as manipulating non-competent hosts densities, or applying insecticide to non-competent hosts have also been explored to control vector-borne diseases, such as in the control of malaria in India (Hewitt and Rowland, 1999; Rowland *et al.*, 2001). Chelbi *et al.* (2008) identified that in *L. major* foci, areas associated with higher rabbit densities close to the household were at a much reduced risk of VL infection, attributable to the preference of the sandflies for non-human hosts and rodent burrows. This may indicate potential for zoonotic control by host manipulation. Chickens have been suggested as possible candidates for the zooprophylactic control of sandflies in the New World but the balance between possible attractive ability to divert sandflies from other hosts against the possibility that chickens may support higher local vector densities has not been explored experimentally or theoretically (Alexander *et al.*, 2002).

1.4. Modelling transmission

Population dynamics modelling is a mathematical tool to aid our understanding of key components of disease transmission and a method by which the complex natural world can be investigated and theoretically manipulated in ways that would be experimentally impossible.

1.4.1. Basic models of infectious disease

The most basic model that can be used to investigate infectious diseases is a compartmental model whereby the infection status of individuals within a host population (susceptible (S), infected (I) or recovered (R) (SIR)) are simulated to show how individuals may move between these classes (Keeling and Pejman, 2008). Deterministic SIR models use rates estimated from biological processes to quantify the number of individuals within different classes, during phases of transient dynamics (epidemic transmission), and equilibrium state dynamics, when the population is either a stable disease-free equilibrium, or experiencing stable transmission (endemic). Basic SIR models are a relatively simplistic means of describing transmission, but can be augmented by the addition of additional infectious classes to improve model realism. For example, the inclusion of a latently infectious class of (exposed) individuals (E) gives rise to SEIR models, which take into account the latent period common to most infections (Anderson and May, 1991; Keeling and Pejman, 2008).

To model vector-borne diseases, transmission cannot be considered as a single host system, and the contribution of the vector must be incorporated into the model. The potential of the vector population to transmit an infection, i.e. to contract and transmit the infection, is known as vectorial capacity (*VC*), and is defined as the daily rate at which future inoculations arise from a currently infective case (Garrett-Jones, 1964b). A variety of models exist for calculating the *VC* of a species, the first of which was put forward by Garrett-Jones (1964b) for malaria.

$$VC = \frac{V(a.\theta_x)^2 p^n}{H_x(-\ln p)} \qquad (1.1)$$

Here, three main processes determine *VC*; the contact rate between vectors and hosts, the biting rate upon the host of interest and the length of the vector infective lifespan. The contact rate between vectors and susceptible hosts is determined within the *VC* equation by the vector density (*V*) per host of interest (H_x), calculated by V/H_x. The biting rate upon the host of interest is represented by $a\theta_x$, where *a* is the vector biting rate, often estimated from the vector's gonotrophic cycle in gonotrophically concordant species, and θ_x is the per vector proportion of bites per host of interest, otherwise know as the Human Biting Index in malaria epidemiology. Biting rate enters *VC* as a squared term because two biting events are needed for successful transmission. Finally, longevity of the vector's infectious period is determined by p^n/-ln*p*. Here, *p* is the daily survival probability and *n* is the extrinsic incubation period, therefore p^n represents the chance of a vector living to become infectious and 1/-ln*p* is the vector life expectancy, together, these determine the length of the infective period. The parameter –ln*p* is therefore the instantaneous vector mortality rate, and often represented by a single parameter (μ_x) (as in Chapter 2).

Values for p and n may be assessed in the laboratory, and p can be estimated from vector parity (Smith *et al.*, 2012), however, to populate even this basic VC model still requires large quantities of data to produce reliable estimates. Therefore, when models of vector borne disease are used to assess the efficacy of interventions they often focus upon the relative change in parameters such as VC, rather than absolute changes in response to interventions (Dye, 1992).

Nevertheless, VC is a critical component of R_0, the basic reproduction number of a disease. This key epidemiological concept, from which VC has been derived by combining its entomological constituents (Garrett-Jones, 1964b), is defined as the number of new cases arising from one case in a fully susceptible host population during infectiousness (Anderson and May, 1991; Macdonald, 1957). The use of mathematics to explore transmission and control of vector-borne diseases was first conceptualised for malaria by Ross (1910), whom developed a set of equations to define a threshold density of vectors at which vector-borne transmission could not be maintained. These ideas underwent subsequent development at the hands of Macdonald (1952) who incorporated biological important entomological components such as vector longevity into the equations, and in particular expanded upon important features of entomological theory, such as basing transmission upon vector feeding cycles and demography, and recognising the importance of a latent period in the vector combined with mosquito survival time (Smith *et al.*, 2012). Thus, the concept and calculation of R_0 was formalised into what is referred to as the Ross-Macdonald model for malaria transmission (Macdonald, 1952).

According to the Ross-Macdonald model, R_0 can be formulated as follows,

$$R_0 = \frac{V(a.\theta_x)^2 b p^n}{H_x(-\ln p) r} \quad (1.2)$$

where the inclusion of *r*, the recovery rate of infected hosts, and *b*, the probability of infection transmission between vector and host, introduce vital parasite related components to the entomological parameters highlighted in *VC* above. Thus, creating a model of vector-borne transmission.

For a vector-borne disease to grow or persist in a given host population the reproduction number must be above or equal to one. Consequently R_0 is one of the major outcomes often calculated from epidemiological models, and the aim of interventions and modelling control options is to understand how to reduce R_0 below one, as this will lead to the eventual eradication of the disease in a given population.

Numerous formulations of R_0 have since been developed for vector-borne infections. These models may differ from one another in order to make use of epidemiological data that may be more readily available to estimate entomological parameters (Dye, 1990; Hasibeder *et al.*, 1992), or may reflect differences in vector or parasite life history in different infections. For example, a similar formulation for R_0 is presented for leishmaniasis in dogs in Chapter 2; however, as leishmaniasis is a fatal infection in dogs the recovery rate (*r*) is not incorporated.

Nevertheless, in order to simulate complex biological systems using models such as R_0 a number of simplifying assumptions are made. These include the assumption that

vector behaviour and dynamics are independent of infection, that no host immunity is acquired, homogeneity of host infectiousness (i.e. no super spreaders), that there is no latent period of infection in the vector, that there is homogeneity of mixing such that the biting rate of vectors is spread evenly across all hosts (dictated by the vector to host ratio) and that vector preference is constant. However, these assumptions are regularly violated in vector transmission (Anderson and May, 1991; Dye, 1990; Rossignol *et al.*, 1986), and in particular when the cases of multiple susceptible hosts populations is considered.

The basic transmission model presented above was formulated to describe the dynamics of malaria, a single host pathogen. Yet, many other vector-borne diseases involve multiple host species, such as in the transmission of *L. infantum,* where humans are susceptible, but dogs are the main reservoir host. Multi-host systems present additional challenges when modelling transmission and must be augmented to reflect the dynamics and relative contribution of each host species to transmission, including their status as a transmitter of disease such as an essential disease reservoir versus non-competent vector maintenance host, and their interaction with the vector population (Streicker *et al.*, 2013). These epidemiologic features have strong implications for observed infection dynamics and possibilities for control (Fenton and Pedersen, 2005). For example, the infectiousness of secondary hosts and their contact rate with the vector population can determine if the parasite is truly multi-host, and therefore if control must be targeted to multiple populations. This is true of the transmission and maintenance of brucellosis in the USA, where many species of large mammal are reservoirs of infection (Haydon *et al.*, 2002). Conversly, if the disease is endemic to only one host population but "spilling over" into another under certain conditions, such as increased proximity with vectors in the case of West Nile

Virus in the USA (Fenton and Pedersen, 2005), this situation would require control targeted to the true reservoir.

VL in the New World involves both a reservoir population (dogs) and a dead-end host population (humans). Therefore, the dynamics of the interaction between these two populations via the vector are likely to be key in determining transmission dynamics and prevalence in these host populations.

Of particular interest when modelling such populations is the critical parameter θ_x which within the R_0 formulation is that of vector preference for host x (Garrett-Jones, 1964a), and is usually assumed to be constant. R_0 is highly sensitive to change in host preference due to the necessity of multiple biting events (one to acquire and one to transmit), and therefore enters transmission models twice. The presence of this squared term in the R_0 formulation indicates R_0 will change nonlinearly with preference, yet, the majority of models assume constant host preference (Dietz, 1982) and do not account for any important heterogeneity in host preference. This is particularly important in multi-host systems where, intrinsically, vectors have a choice of blood source. Dynamic change in host choice responses have the potential to seriously influence transmission dynamics, especially when the available hosts are differentially infectious to sandflies (Fenton and Pedersen, 2005). For example if one host population is an infectious reservoir and another is dead-end host, as in the case of New World VL, then any change to host choice between these two is likely to result in complex nonlinear transmission dynamics which need to be incorporated if transmission and control are to be understood (Fenton and Pedersen, 2005).

Similarly, biting rate upon hosts is also influenced by the density of the host of interest (H_x) is expressed as a relative abundance, therefore changes in abundance of non-competent host or dead-end hosts will also influence biting rate per host, and therefore R_0 nonlinearly (Woolhouse *et al.*, 2001). However, that is again assuming that only relative host densities and a constant preference determine biting rate. Alternatively, foraging theory would indicate that the gains in fecundity from feeding on a particular host species would be traded-off against the costs, such as host defensive behaviour, leading to dynamic preferences for different species depending upon the vector density per host (Kelly and Thompson, 2000), which can too lead to nonlinear changes in R_0 with host ratios and vector density. Therefore, homogeneity in distribution between available hosts of each species is a critical assumption that is likely to be invalid in the case of sandflies, which follow a highly aggregated distribution (Kelly *et al.*, 1996), and additional investigation of preference dynamics and modelling may be required to elucidate.

Overall, therefore it is important to challenge basic assumptions upon which modelling of transmission is based and where possible incorporate additional elements of host and vector dynamics into systems in order to increase model realism and subsequent usefulness. In the case of multi-host transmission this is likely to include exploration of biting dynamics, however, there are various other components of vector-borne disease, for example, incorporation of vector-parasite interactions (Killeen and Smith, 2007), spatio-temporal dynamics (Tanser *et al.*, 2003) or stochasticity (Macdonald *et al.*, 1968) which could also be incorporated to improve models.

1.4.2. Models of Leishmania *transmission*

In contrast to malaria, relatively few attempts have been made to model leishmaniasis, consequently there is much scope for improvement in *Leishmania* models and predictions regarding the effect of interventions.

Most notably, Dye (1996) developed the definitive VL model on which most subsequent models appear to be based (Courtenay *et al.*, 2002b; Palatnik-De-Sousa *et al.*, 2004; Reithinger *et al.*, 2004). Here, canine reservoir dynamics are modelled within an adjusted SEIR framework, including vectors via vectorial capacity, and human infection rate as a function of the number of infected vectors. In this framework, as with other diseases, increased vector mortality, induced by insecticide, is predicted to have the greatest impact upon *Leishmania* prevalence. However, the model is limited by its simplistic simulation of transmission dynamics, failing to incorporate vector population and preference dynamics, dynamics of alternative hosts, or the contribution from other non-competent hosts such as chickens. Additionally, the impact of interventions such as insecticide was crudely formulated as a function of vector mortality (Dye, 1996), when it is likely that changes to vector density and repellent activity will carry consequences for host choice, which are particularly important in the transmission of multiple host pathogens.

Alternative models have incorporated vector infection dynamics, but continued to ignore the contribution of alternative hosts to transmission dynamics or possible diversion effects of insecticide via their effect upon vector biting preferences (e.g. Reithinger *et al.* (2004)). The inclusion of differential equations describing

transmission with both vector and multiple host species has been developed for the study of VL in Africa, where it is expected that both humans and zoonotic reservoirs may be infectious to the vector (Elmojtaba *et al.*, 2010), although constant vector preferences between hosts of differing infectivity is assumed.

Intrinsically, infection rates must be based upon sandfly biting rates, biting preferences and transmission probabilities. Chaves *et al.* (2004) include these together as a single term for infection rate which not only assumes that transmission probabilities between flies and reservoir hosts are identical irrespective of direction but also that biting preferences are fixed and constant. However, Chaves *et al.* (2007) goes on to acknowledge the effect of the addition of new hosts species of differing infectivity can result in the nonlinear dilution of R_0 in cutaneous leishmaniasis. Something that could be highly relevant in VL modelling where the relative contribution made to transmission by individuals of varying disease status (clinical VL, PKDL or asymptomatic) may differ. However, Chaves *et al.* (2007) does not directly consider the densities of different host types *per se*, but this is an obvious application of the model, yet preference and infectivity of a species is fixed, when in reality Especially as there is evidence to suggest that sandflies are disproportionately attracted to infected hosts (O'Shea *et al.*, 2002)

From the above it is clear that there are multiple aspects of *Leishmania* transmission that have not yet been modelled or brought together into one model of transmission. In particular, as highlighted by Harvey *et al.* (1988), epidemiological models often fail to take into account aspects of host and vector ecology; as such leishmaniasis needs to now be modelled including all reservoirs hosts, maintenance hosts *and*

vectors of disease, before investigating the impacts of control measures. However, investigations demonstrating how the different host species interact with each other and vectors to determine such dynamics are lacking, making it impossible to include such dynamics in models. It is therefore a main aim of this thesis to investigate such fundamental relationships in order to improve understanding of transmission in order to aid parameterisation of *Leishmania* transmission models and prediction of control.

1.5. Aims and Objectives

The general project aims are as follows:

1. Investigate the relationship between *L. infantum* infection in dog and human populations in an endemic area
2. Investigate possible mechanisms behind the prevalence relationships (identified in Objective 1, above) using mathematical models.
3. Establish an unbiased means of investigating vector-biting preference in the field.
4. Investigate the patterns of vector-biting preference in relation to vector density.
5. Investigate the patterns of vector-biting preference in relation to seasonality.
6. Investigate the patterns of vector-biting preference in relation to host density.

1.5.1. Approach

In order to address the above aims the thesis is structured as follows. Chapter 1 provides commentary on the epidemiology of leishmaniasis, current understanding of transmission and options for control. Chapter 2 contains a modelling study based on published data, following a meta-analysis of leishmaniasis host prevalence relationships. The results from this work were presented in poster form at British Society for Parasitology (12th-14th April 2011) and Royal Society of Tropical Medicine and Hygiene (19th-21st September 2012) conferences. The findings stimulated the experimental designs in the following three chapters. In Chapter 3, the methods are described, including the development and optimisation of trapping protocols upon a range of domestic hosts, to permit the accurate assessment of vector preferences in the field. In Chapter 4, the relationships between vector biting rates and preference for different key peridomestic hosts over a period of 5 months are presented. In Chapter 5 the relationship between host density and vector biting preference is presented. In Chapter 6, the relationships demonstrated in the Chapters 4 and 5 are explored in greater depth using statistical modelling of the data presented in Chapter 5. Finally, the general findings, implications, limitations and possible future research options leading on from this study are discussed in Chapter 7.

Chapter 2: **Nonlinearities in zoonotic visceral leishmaniasis transmission**

2.1. Introduction

In Latin America the causative agent of VL is the obligate intracellular parasite *Leishmania infantum*. *L. infantum* is principally a zoonotic infection, whereby humans are a "dead end" with respect to transmission and the infection is instead maintained within an animal reservoir, namely domestic dogs (Courtenay *et al.*, 2002a).

VL is transmitted between dogs and to people by the bite of infected female sandflies of the *Lutzomyia longipalpis* species complex (Maingon *et al.*, 2003; Ward, 1983; Ward *et al.*, 1986). Therefore, local vector presence is a prerequisite for transmission in both populations (Caldas *et al.*, 2002), but there is significant variation in the infection rate between regions. This is likely to reflect differences in local vector abundance and host population composition which impact upon infected vector biting rate (Dye *et al.*, 1993). However, it is proposed here that differences between regions may also be related to variation in host biting preferences between locations.

As discussed in Chapter 1, *Lu. longipalpis* are eclectic in their feeding preference and readily feed upon a wide variety of hosts within the peridomestic environment (Deane and Deane, 1962; Morrison *et al.*, 1993b). Nonetheless, natural host preference appears to occur as innate function of hosts biomass (Quinnell *et al.*, 1992). This is most likely because larger animals produce more kairomone, a key odour attractant of sandflies in the lab (Morton and Ward, 1989). Local abundance

and distribution of females flies is also likely to be influenced by the distribution of male flies (Campbell-Lendrum *et al.*, 1999a) which form lek-like aggregations in association with hosts (Jones and Quinnell, 2002). Here, the males produce a sex pheromone which works in synergy with host odour and attracts females over distance (Morton and Ward, 1989; Quinnell and Dye, 1994b).

Host density has also been shown to be associated with fly distribution and density (Fernandez *et al.*, 2010), with multiple studies having identified specific host species, in particular chickens, dogs and number of people per household as significant risk factors for human (Badaro *et al.*, 1986; Dye and Williams, 1993; Fernandez *et al.*, 2010; Quinnell and Dye, 1994a) and canine infection (Miles *et al.*, 1999; Moreno *et al.*, 2005).

However, it is the number of infected reservoir animals that is key to transmission. Oliveira *et al.* (2001) identified a statistically significant association between the number of seropositive dogs and number of clinical human cases, and also highlighted how peaks in canine seropositivity and human cases are closely associated in space and time. Unfortunately, clinical burden gives a poor indication of the underlying rate of human infection (Michalsky *et al.*, 2009), and there have not been consistent reports of a relationship between the rate of exposure in humans and canine infection (Caldas *et al.*, 2002; Fernandez *et al.*, 2010; Moreno *et al.*, 2005), with Moreno (2005) failing to identify clinically sick dogs as a risk factor for infection in humans.

Overall, the relationship between infection rate in reservoir, accidental hosts (humans) and vector populations remains poorly characterised. The general assumption therefore remains that that infection rates in susceptible host populations are linearly related to one another (Dye, 1996), and therefore that halving the infection rate among dogs would result in a halving of the infection rate among humans. Such assumptions are poorly substantiated in the literature and do not take into account potentially important features of vector dynamics which may have important implications for understanding transmission and control.

2.2. Aims

In order to improve understanding of transmission it is necessary to elucidate the relationship between infection rate in canine reservoir and human populations. To achieve this, the following objectives must be met:

1. Quantify the relationship between prevalence of infection in dog and human populations using comparable field data.
2. Investigate possible underlying processes within the human and dog population prevalence data through the development of a mathematical model of transmission.

2.3. Methods

2.3.1. Data collection

Data for this investigation were collected from a wide range of published and unpublished sources.

The majority of data come from two separate studies into VL prevalence carried out on the Island of Marajó, Brazil (see Figure 2.1). The first study began in 1989 whereby serological surveys of the dog population were carried out annually for 5 years using Immunofluorescence Antibody Test (IFAT) to test a total of 1,327 samples from 23 villages (Courtenay *et al.*, 1994) (see Appendix B for experimental details). Survey of the human population by Montenegro Skin Test (MST) was then carried out a year later in 1995 for 11 of these villages (Quinnell, unpublished). Due to the differences in collection locations, dates and survival of dogs, it was only possible to match canine data from 10 villages collected in 1994 to the human surveys of 1995 for this investigation. In 2004 a second study began; 18 Marajó villages were surveyed for canine infection, this time by Enzyme Linked Immunosorbent Assay (ELISA), and again for human infection by MST the following year (Courtenay, unpublished).

2.3.2. Sandfly survey

A survey of peridomestic sandfly density was performed in July-August 1992 in 172 houses in 14 communities as previously described (Quinnell and Dye, 1994a).

Briefly, two CDC light traps (CDCLT) were set overnight, one positioned inside the house, the other in the animal shed (usually poultry shed) or near to the animals if a shed was absent. Previous surveys in the study villages consistently demonstrated that >93% of peridomestic CDCLT light trap sandfly catches are *Lu. longipalpis* (Courtenay *et al.*, 2007; Kelly and Dye, 1997; Lainson *et al.*, 1990). Species identity was periodically checked by visual inspection of male flies. Due to the overdispersed nature of sandfly count data the geometric mean fly density per household per village was used to indicate the average vector density by village.

2.3.3. Published Data Collection

To construct the largest possible comparable dataset of *L. infantum* prevalence in dogs and humans for South America additional prevalence data were collected from published and unpublished sources via systematic search in English of Web of Knowledge, CENTRAL, BIOSIS and WHO trial databases. Key search terms used in combination were leishmaniasis, *Leishmania infantum*, *Leishmania chagasi*, *Lutzomyia longipalpis*, New World, prevalence, dogs, humans, infection, serology, ELISA, IFAT, Leishmanin Skin Test (LST) and MST. In total, these returned 26 unique hits, from which only studies which used comparable methods of both human and dog population diagnostic survey were selected. This meant that only surveys using canine serology and human skin tests were of interest. These techniques are analogous to one another in their respective host species, i.e. both exhibit long-term positivity and therefore indicate the prevalence of exposure and asymptomatic infection in both populations (Table 2.2). This is important as asymptomatic dogs may significantly contribute to transmission in some settings (Moreno and Alvar, 2002), and clinical signs are a poor indicator of infection status. Furthermore, the

prevalence of clinical signs in humans is a poor indicator of infection risk and is likely to suffer underreporting in the public records. For studies where interventions took place only baseline prevalence was noted. Sources are summarised in Table 2.1.

1. Island of Marajó, Pará State (Courtenay, unpublished; Quinnell, unpublished).
2. Brotas, Ceará State (Evans *et al.*, 1992).
3. Monte Gordo, Bahia State (Cunha *et al.*, 1995).
4. Pancas, Espirito Santo State (Falqueto *et al.*, 2009).

Figure 2.1: Map of South America, with inset of Brazil to indicate the locations from which data on human and canine *Leishmania* infection prevalence data originated. Image adapted from Bing Maps (Microsoft Corporation 2013, Nokia 2013).

Table 2.1: Summary of human and canine *Leishmania* prevalence data, gathered from published and unpublished sources.

Data source	Details	Canine test	Human test	Prevalence in dogs % (+ve/total)	Prevalence in humans % (+ve/total)
Quinnell (unpublished)	10 villages on Marajó, Brazil. 1994.	IFAT	MST	40.5 (43/107)	51.7 (195/377)
Courtenay (unpublished)	18 villages on Marajó, Brazil. 2004.	ELISA	MST	55.5 (244/440)	41.2 (1243/3016)
Cunha *et al.* (1995)	Monte Gordo, Bahia, Brazil. Collected 1991.	IFAT	MST	46.0 (7/15)	32.0 (48/152)
Evans *et al.* (1992)	3 municipalities near town of Brotas, Ceara, Brazil. Collected 1987.	ELISA	MST	47.7 (94/197)	33.4 (193/578)
Falqueto *et al.* (2009)	Pancas, Espirito Santo, Brazil. Collected 2003-4.	ELISA	MST	57.0 (32/109)	33.2 (92/277)

Table 2.2: Summary of the test types used and their reported sensitivity and specificity.

Test type	*Details*	*Sensitivity*	*Specificity*	*Source*
IFAT	Serological test, current test of choice for testing dogs in Brazil	72-100%	52-100%	(Romero and Boelaert, 2010)
ELISA	Serological test, used in dogs and humans.	70-100%	86-100%	(Romero and Boelaert, 2010)
MST	Testing the hypersensitivity of human to Leishmania antigens, to confirm exposure to parasites	50-100%	Not specific for current VL (picks up historical)	(Berman, 1997; Weigle et al., 1991)

2.3.4. Age and sex adjustment

Raw prevalence data were adjusted by age and sex for both the human and dog populations due to identified differences in the stratum-specific infection rates by age and sex, in order to identify the age and sex adjusted proportion of exposed individuals from of each host population This was done using the indirect standardisation methodology (Kahn and Sempos, 1989; Kirkwood and Sterne, 2003). Estimates from this method are robust to small sample sizes, making it appropriate for dealing with the small canine populations sampled in a number of the study villages. In brief, this standardisation methodology used age and sex specific rates, as determined from a standard population, to standardise the crude stratum specific prevalence for each village, and thus remove bias in our prevalence estimates caused by differences in age and sex distribution between villages. The total Marajó dog population data from 2004 and the Marajó human population data from 2005 were used as the standard reference populations for dogs and humans respectively. Dogs were categorised into the following approximately equally sized age groups of 0-0.5, 0.5-1, 1-2, 2-4, 4-19 years. Humans were also sorted into equally sized age categories of 0-1, 1-6, 6-12, 12-20, 20-35, 35-50, 50-100 years.

2.3.5. Analysis

Firstly, it was attempted to calculate incidence from age prevalence curves by maximum likelihood (Williams and Dye, 1994), however, these calculations did not produce reliable estimates of incidence and therefore adjusted estimates of prevalence were used in further analyses. These prevalence data were analysed using

generalised linear models (GLM) for associations between human and dog prevalence at the village level after each data point was weighted by human and dog population size, and differences between serological test type and data source were taken into account as fixed effects. Analysis was carried out on the total dataset and then repeated for a restricted dataset excluding villages with fewer than 10 dogs and 20 humans sampled, or villages where there highly biased age distribution meant it had not been possible to obtain an estimate of incidence.

In order to investigate possible nonlinearities in the data GLM analyses with and without quadratic terms were fitted to the data and compared. Akaike information criterion (AIC) (Akaike, 1974) and likelihood ratio tests were used to identify which model gave the best fit to the data. All analysis were carried out in STATA 11 (StataCorp, 2010) and tests were considered significant at the $P=0.05$ level.

2.3.6. Dynamic Model formulation

A minimalistic transmission model was formulated to describe the basic transmission cycle of zoonotic VL and investigate the relationship between prevalence in dog and human populations, based on Dye (1996).

The most basic model contains only reservoir hosts and vectors classified into either susceptible or infectious classes, with the proportion of infected dogs (y) being determined by rates of canine infection and infected canine mortality (μ_D). The transmission rate to dogs is intuitively understandable as being dependent upon the biting rate of vectors (a), the proportional preference for dogs (θ_D), the probability of

successful transmission from sandfly to dog (α), the proportion of infected vectors (v) and vector density (V). Similarly, the infected proportion of sandflies (v) is dependent upon sandfly mortality rate (μ_V) and the successful biting rate upon infected dogs, determined fly biting rate (a), the proportional biting preference on dogs (θ_D), proportion of infective dogs (y), and the probability of transmission from dog to sandfly (σ). It is assumed that canine hosts and vectors are born susceptible and do not recover. These processes are formally represented below (equation 2.1).

$$\frac{dy}{dt} = a\theta_D \alpha v \frac{V}{D}(1-y) - \mu_D y$$

$$\frac{dv}{dt} = a\theta_D \sigma y(1-v) - \mu_V v \tag{2.1}$$

When infection rate in these populations is at equilibrium these equations can be solved to express the basic reproductive ratio of canine infection, R_0,

$$R_{0^D} = \frac{(a\theta_D)^2 \alpha\sigma \frac{V}{D}}{\mu_D \mu_V} \tag{2.2}$$

and the equilibrium prevalence for the canine (y*) and vector (v*) populations.

$$y^* = \frac{(R_0 - 1)}{R_0} \frac{a\theta_D \alpha \frac{V}{D}}{(\mu_D + a\theta_D \alpha \frac{V}{D})} \tag{2.3}$$

$$v^* = \frac{(R_0 - 1)}{R_0} \frac{a\theta_D \sigma}{(\mu_V + a\theta_D \sigma)} \tag{2.4}$$

Note that the biting rate per fly upon dogs (equation 2.4) is a squared function, indicating the necessity for two successful biting events upon dogs to complete transmission.

Once prevalence in the vector population is known, it is possible to calculate the instantaneous biting rate to the human population (λ_H) from the biting rate and proportional preference for humans (θ_H) in addition to the infected vector density.

$$\lambda_H = a\theta_H V v^* \tag{2.5}$$

Since prevalence is a cumulative but saturating function of infection rate it is possible to calculate MST prevalence among humans (h^*) using equation 2.6, taking into account the probability of infection and detection of infection in humans (π_0), human life expectancy (L) and the rate of loss for MST positivity (π_1). This is the solution of the age related differential equation for MST positivity in the human population, assuming all rates are constant with age.

$$h^* = 1 - \frac{\pi_1}{\pi_1 + \pi_0 \lambda_H} \frac{(1 - \exp^{-L(\pi_1 + \pi_0 \lambda_H)})}{1 - \exp^{-L\pi_1}} \tag{2.6}$$

MST positivity is known to be long lived but not lifelong in humans, therefore loss of positivity is highly relevant to modelling MST prevalence. Parameter π_0 acts as a correction factor upon human MST prevalence for the combined effects of test sensitivity as well as transmission probability. This parameter subsumes all the parameters relating to human MST positivity, namely, transmission rate to humans and sensitivity of MST.

2.3.7. Estimation of biting preference

Using this basic transmission model in conjunction with prevalence and vector density field data obtained for 11 surveyed villages for which vector density estimates were also available, the values for vector biting preferences (θ_D and θ_H) that gave rise to observed prevalence rates in human and canine field populations were derived via an iterative process. All survey populations were assumed to have reached equilibrium prevalence, as only pre-intervention population data was selected for these analyses.

In all instances where the calculated preferences did not sum to 1 it was assumed that there must also have been a preference for alternative hosts in effect. In this case the alternative host was assumed to be chickens, and the proportional preference for chickens (θ_C) was calculated following re-arrangement of the following equation.

$$1 = \theta_D + \theta_H + \theta_C \tag{2.7}$$

All modelling work was carried out using MATLAB v.10.

2.3.8. Parameterisation and Assumptions

Where possible model parameter values are determined from published literature and assumed to be constant (listed in Table 2.3). However, the probability of transmission from an infected fly to a susceptible human (γ) has not been assessed experimentally due to ethical considerations. Since the probability of human

infection and detection of infection are collinear, and neither is perfectly measured, they are subsumed into one process, (π_0).

Similarly, longevity of MST positivity is difficult to determine as skin test antigen sensitivity varies over time, thus confusing results (Bern *et al.*, 2006). Therefore, the rate of loss of MST response is potentially highly variable, but is estimated from the current data. It is considered to provide a long-term but not lifelong response, estimated from the age related MST response data of Quinnell (unpublished) and Courtenay (unpublished) to last an average of 40 year.

Table 2.3: Variables and parameter values used in the basic *Leishmania* transmission model and statistical models.

Symbol	Parameter or variable	Value	Source
μ_D	Death rate of infected dogs	0.003006 day^{-1}	(Courtenay, 1998)
μ_V	Death rate of vectors	0.41938 day^{-1}	(Dye, 1996)
L	Human life expectancy	26535.5 days	(World Bank, 2010)
a	Daily biting rate of sandflies	0.333 day^{-1}	(Dye *et al.*, 1991)
α	The probability of an infected sandfly transmitting to a dog	0.321	(Reithinger *et al.*, 2004)
σ	The probability of an infected dog transmitting to a sandfly	0.107	(Courtenay *et al.*, 2002b)
γ	Probability of an infected sandfly transmitting to a human	Unknown. Subsumed into π_0	
π_0	Probability of an infected sandfly transmitting to a human and being detected by MST	0.1605	Unknown. Assumed to be half transmission probability from sandfly to dog.
π_1	Rate of loss of MST response	6.85×10^{-5} day^{-1}	Estimated from Quinnell (unpublished) and Courtenay (unpublished)

V	Absolute vector density		Data
$\overline{V}$	Relative vector density		Data
V_S	Sum of vector densities across hosts		Data
V_{CDC}	Number of vectors per CDCLT		
H_x	Density of host x		
D	Dog density		
ECD	Effective chicken density		
C_t	Number of chickens at time *t*		Data
y*	Proportion of dogs infected at equilibrium		Data
h*	Proportion of humans infected at equilibrium		Data
v*	Proportion of female sandflies infected at equilibrium		
λ_H	Instantaneous biting rate to humans		
θ_D	Biting preference of sandflies for dogs (proportion of bloodmeal of dog origin)		
θ_H	Biting preference of sandflies for humans (proportion of bloodmeal of human origin)		
θ_C	Biting preference of sandflies for chickens/alternative hosts (proportion of bloodmeal of other host origin)		
R_0	Basic reproduction number of leishmaniasis in dogs		
FOI_D	Proxy for force of infection to dogs		
FOI_H	Proxy for force of infection to humans		
e	Relative trap efficiency		
κ	Decay rate in chicken attractiveness	0.8 day^{-1}	Unknown.
t	Time		
β	Regression coefficient		
c	Intercept on the Y axis		
z	Z-statistic		
T	T-statistic		
P	P-value		

2.4. Results

2.4.1. Nonlinear change in human prevalence

Raw data from published and unpublished surveys of dog populations using IFAT or ELISA and human populations using the MST were used to investigate the relationship between humans and dog infection rates (data listed by point with CI in Appendix C). Over 6 independent studies spanning 18 years, 4,875 people and 1,008 dogs were tested by MST and IFAT/ELISA respectively for *Leishmania* infection (Table 2.1). Of the surveyed hosts, 37.7% of people and 46.6% of dogs tested positive for *Leishmania* exposure.

After age and sex adjustment of the prevalence rates, controlling for differences in serological test and data source and weighting points by sample size, analysis of the full and restricted datasets reveals a nonlinear relationship between *Leishmania* prevalence in dogs and humans (Figure 2.2). Highly significant linear ($\beta=13.14$, $z=8.24$, $P<0.0001$) and quadratic terms ($\beta=-8.28$, $z=-7.48$, $P<0.0001$) within the model and a reduction in the AIC following the addition of the quadratic term (without quadratic term AIC=11.96, with quadratic term AIC=10.27), demonstrate this significant nonlinearity. This nonlinear trend is especially marked in the restricted dataset (Figure 2.3). It appears that high rates of infection in dogs do not induce correspondingly high rates of exposure in human populations. Here again, increases in prevalence among dogs are initially associated with increasing human prevalence ($\beta=25.08$, $z=8.02$, $P<0.0001$), and associated with a significant decrease in infection in people latterly ($\beta=-18.34$, $z=-7.32$, $P<0.0001$). The inclusion of the

quadratic term in the restricted model also improves models fit, as indicated by AIC values (without quadratic term AIC=12.26, with quadratic term AIC=9.46).

Further analysis of the data for which vector density estimates are available reveals a significant linear relationship between vector density, as estimated from indoor collections, and canine prevalence (*β=0.14, z=3.08, P=0.002*). However, it also shows a significant nonlinear relationship between geometric mean household sandfly density and prevalence among humans, with both a significant increase (*β=0.25, z=4.49, P<0.0001*) and decrease with vector density (*β=-0.007, z=-3.18, P=0.001*). This suggests that nonlinearity in the relationship between human and canine prevalence may be due to variation in biting rate upon different host types with vector density.

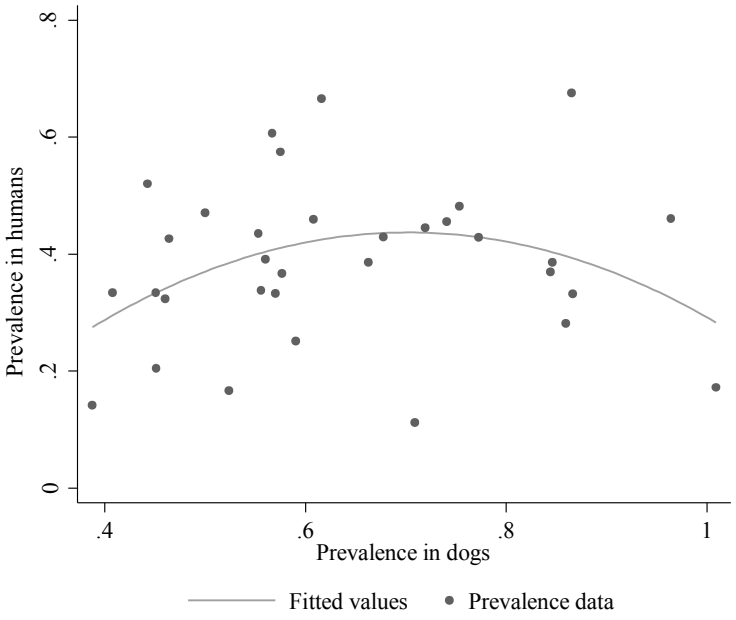


Figure 2.2: Prevalence of humans testing positive for *Leishmania* by MST in association with the corresponding prevalence of *Leishmania* infection in the local reservoir population, as estimated from serological tests (full dataset).

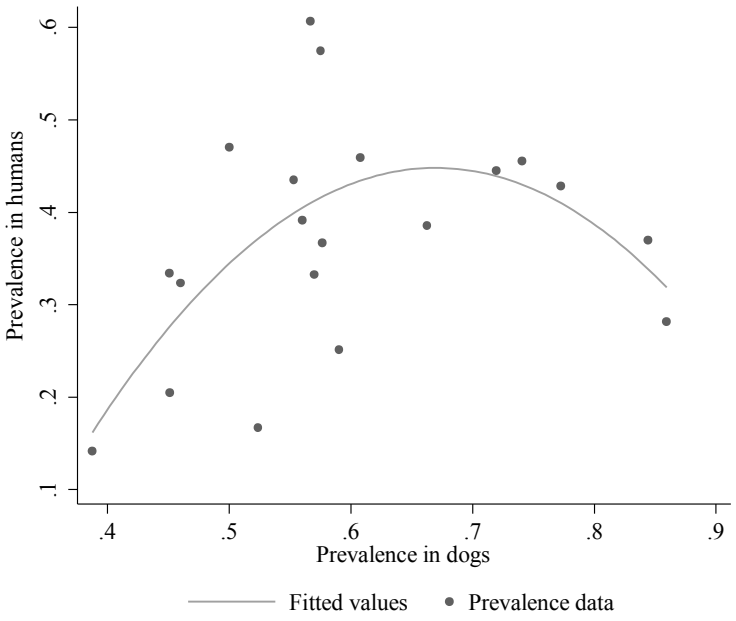


Figure 2.3: Prevalence of humans testing positive for *Leishmania* by MST in association with the corresponding prevalence of *Leishmania* infection in the local reservoir population, as estimated from serological tests. Note this plot represents the restricted dataset only; only sites sampling 10 or more dogs, 20 or more humans and giving a reliable estimate of incidence are included.

2.4.2. Vector density and biting preference

Using a minimalistic mathematical model, village level prevalence and vector density data (when both were available) the prevalence of infection among the vector population (v^*) was calculated. Using this estimate the corresponding biting preference of the vector population for dogs (θ_D) could be calculated by solving for equation 2.3 and 2.4, assuming host ratios were constant. Similarly, the preference for humans (θ_H) could also be solved using this available data using equations 2.5 and 2.6, and the remaining proportional preference was attributed to chickens (equation 2.7). These model-derived estimates of host preference by location were then used to further explore host preference and vector density relationships.

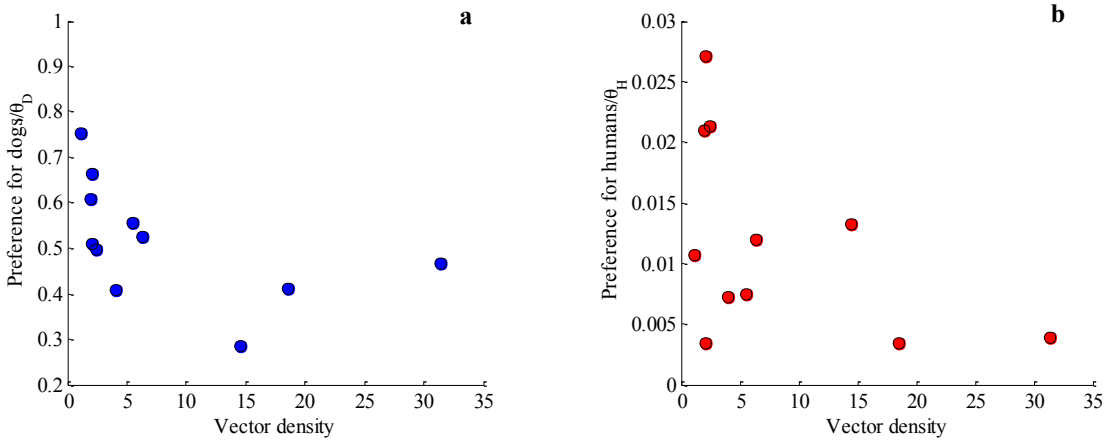
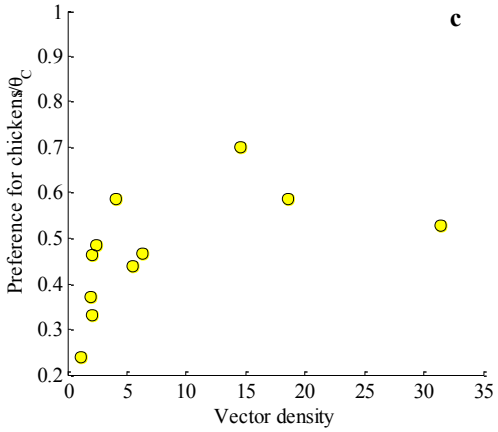
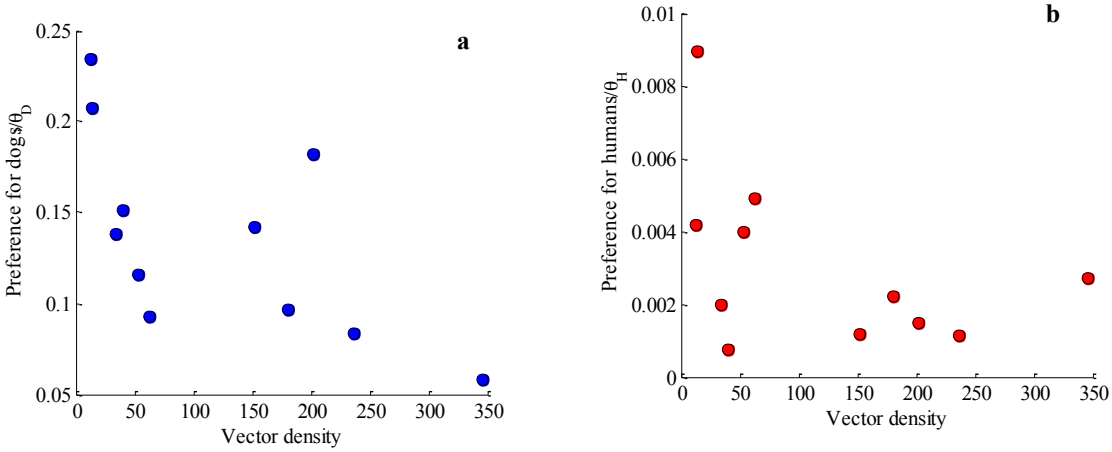


Figure 2.4: The village level model-derived vector preference for (a) dogs, (b) humans and (c) chickens with geometric mean indoor household vector density.





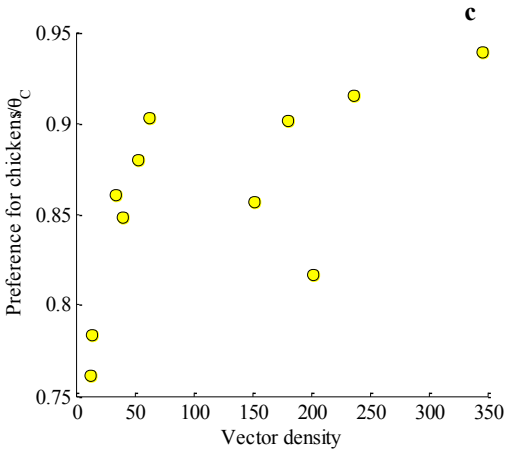


Figure 2.5: The relationship between village level model-derived vector preference for (a) dogs, (b) humans and (c) chickens and average total (indoor and outdoor) household vector density.

Using vector preferences calculated using indoor vector density estimates it appears that fly biting preference for dogs and humans reduces with vector density, whilst the preference for alternative hosts such as chickens correspondingly increases (Figure 2.4). This indicates a possible density-dependent element to host preference. Moreover, shifts in preference with vector density appear to be strongly nonlinear, with preference tending towards non-competent hosts (for leishmaniasis) at higher densities, where it then plateaus. This may indicate that biting preference behaviour among sandflies is most sensitive to change in vector density at lower vector densities, and becomes more stable at higher densities.

Similarly, when using a total household average vector estimates, determined from combined indoor and outside sandfly collections, the relationships between vector density and preference remain highly nonlinear. Again, reduction in the preference for humans and dog corresponds to nonlinear increase in the preference for chickens (Figure 2.5), although the variation around this trend appears to be more than when preference is calculate from indoor vector densities.

Results also indicate that feeding preference for humans is generally low, with only 0.5-3.5% of vectors feeding on humans. This still corresponds to a range of infection rates, from 10-60%, indicating that human populations may be sensitive to small changes in biting preference. This may be because human longevity ensures that even low biting rates result in a large amount of accumulated infection, and magnifies small differences in basal host preference. However, the preference for humans does not appear to be well correlated with preference for dogs or chickens (Figure 2.6a,c and 2.7a,c). Therefore there is little evidence to suggest which host

flies preferentially switch to as preference for humans changes with vector density (as shown in Figure 2.4, 2.5).

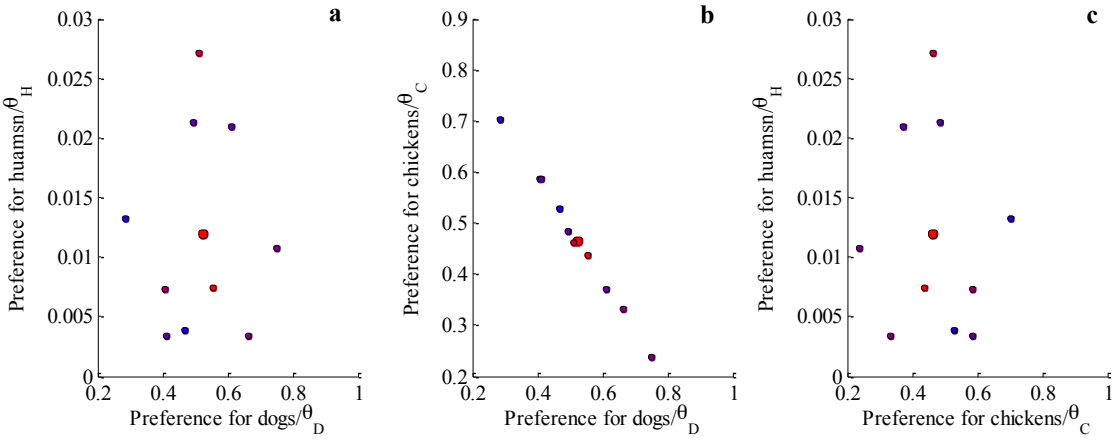


Figure 2.6: The relationship between model-derived proportional preference (a) for humans and dogs; (b) for dogs and chickens and (c) for humans and chickens, calculated using indoor vector density only. Size of data point indicated size of dog population, intensity of red colour indicates size of human population.

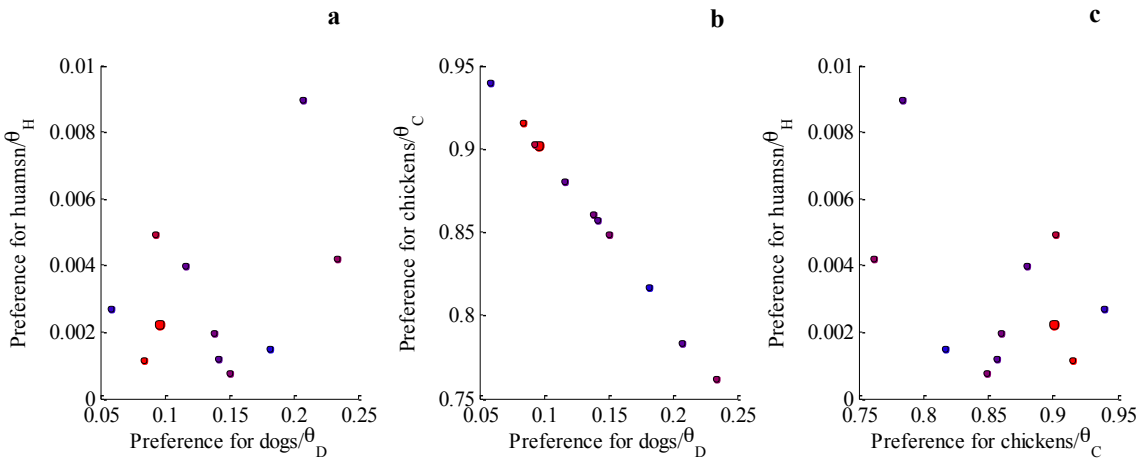


Figure 2.7: The relationship between the model-derived proportional preference (a) for humans and dogs; (b) for dogs and chickens and (c) for humans and chickens, calculated using total vector density per household. Size of data point indicated size of dog population, intensity of red colour indicates size of human population.

By comparison, preference for chickens and dogs appears to be very strongly negatively related (Figure 2.6b, 2.7b). This almost perfectly proportional relationship may indicate that any change in the preference for chickens (θ_C), due to effects of vector or host density, may also impact upon the preference for dogs, however, due to the nature of the calculation for the preference for chickens (equation 2.7), this apparently inverse relationship is unsurprising given the very low values of human preference (θ_H), making interpretation very difficult.

2.4.3. Vector density and transmission rates

Utilising the above vector densities and inferred proportional preferences, epidemiologically relevant outcomes of effective biting density (number of bites per host type once preference is accounted for) and transmission rates to each host were calculated. These reveal that effective biting density upon each host increases linearly with vector density, despite nonlinear changes in biting preference for each host, due to the combination of both increasing proportional preference and absolute vector density (Figures 2.8a and 2.9a). Yet, the reduction in preference for humans with vector density ensures that biting rate on humans remains consistently low even when vector density is high. The rate of transmission to dogs and humans increases with vector density despite corresponding reductions in preference (Figures 2.8b, c), however, nonlinearities in preference with fly density appear to translate into possibly nonlinearly increasing transmission rate to humans (Figure 2.8c).

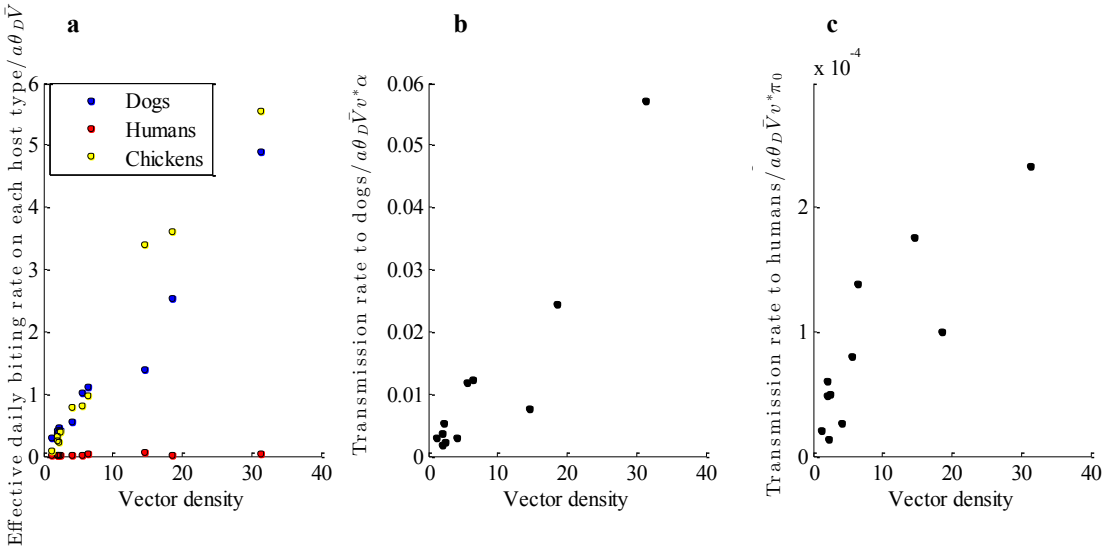


Figure 2.8: The relationship between average indoor vector density and (a) effective biting rate upon each host type, (b) the transmission rate to dogs, (c) the transmission rate to humans.

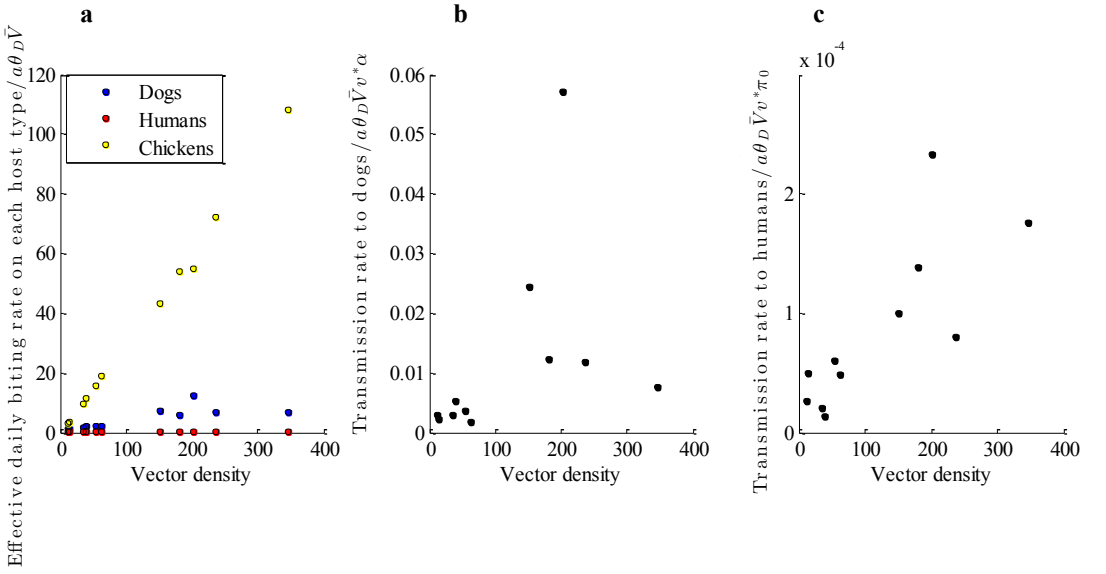


Figure 2.9: The relationship between average total vector density and (a) effective biting rate upon each host type, (b) the transmission rate to dogs, (c) the transmission rate to humans.

By contrast, when effective biting rate and transmission rate are calculated taking outdoor vector density into account, different relationships between vector densities, effective biting and transmission rates emerge (Figure 2.9). Firstly, the effective biting rate on chickens is consistently higher than for the other hosts, and increases dramatically with fly density. Secondly, the transmission rate to people appears to

increase linearly, while the transmission to dogs shows a possible nonlinear relationship, although this is not very clear. These relationships need further consideration, as depending on the measure of vector density (indoor/outdoor) they offer alternative interpretations of how the estimated host preference translated into transmission potential to each host, and resulting prevalence.

2.5. Discussion

From analysis of published and previously unpublished data the relationship between the prevalence of VL exposure in dogs and humans appears to be statistically significant and nonlinear, with prevalence in humans initially increasing, but then decreasing with prevalence in dogs. As explored by estimation of associated host preferences, this nonlinearity is potentially due to variation in vector biting preference across the available hosts with vector density.

This investigation represents the first attempt to document and explore the relationship between exposure to *L. infantum* infection in dogs and humans measured by comparable diagnostic methods. These data challenge a basic assumption prevalent in the VL literature, that *Leishmania* infection prevalence in the key reservoir populations (dogs) and the susceptible populations of interest (humans) follow simple, linear relationships with one another (Courtenay *et al.*, 2002b; Dye, 1996; Reithinger *et al.*, 2004). The nonlinearity is also in contrast to the linear trend reported between clinical VL cases in humans and canine seroprevalence by Oliveira *et al.* (2001). However, a cross section of clinical cases is a highly unreliable measure of VL exposure with anywhere between 4-30:1 cases being asymptomatic (Michalsky *et al.*, 2009), and is therefore a poor indicator of true infection risk.

The nonlinear changes in host preference in VL may well be caused by pheromone mediated aggregation behaviour of the principal VL vector, *Lu. longipalpis* (Kelly and Dye, 1997). Here, males aggregate upon stationary hosts and emit sex pheromone, which, in synergy with host odour attracts blood feeding females to the host in order to complete their reproductive cycle (Bray *et al.*, 2009; Bray and Hamilton, 2007b; Morton and Ward, 1989). Pheromone also attracts more males to the location, which then too commence pheromone production. This positive feedback mechanism and the attraction of females to leks as a function of lek size (Kelly and Dye, 1997), ensures that *Lu. longipalpis* distribution is highly aggregated (Kelly and Dye, 1997; Ximenes *et al.*, 1999), and is the possible mechanism underlying density-dependent host preference. In summary, at greater vector densities increased pheromone production on non-competant hosts, such as chickens, may contribute to greater aggregation effects, which may contribute towards the tendency to aggregate and feed upon chicken hosts, rather than dogs and humans.

The near proportional relationship between preference for dogs and chickens is likely to be a reflection of a model limited to a three-host choice where one host (humans) receives very few bites. However, as preference for all three hosts types vary consistently with vector density, it might be anticipated that there are relationships between the preferences are consistent too. The relationship between preference for dogs and alternative hosts (assumed to be chickens) may therefore also indicate that the majority of sandfly activity shifts primarily between outdoor locations (dogs and chickens), rather than leks commencing on humans. Alternative hosts such as chickens may be preferentially aggregated upon with vector density due to their

accessibility within poorly constructed chicken sheds (Quinnell and Dye, 1994b), abundance and biomass (Quinnell *et al.*, 1992), and putative lower defensive behaviour in comparison to other hosts. In particular, chickens sleep at crepuscular biting hours and may therefore be less defensive while dogs and humans remain active for longer. By contrast, given their biomass and abundance, hosts such as humans appear to be fed upon relatively infrequently. This is also largely a result of humans being active during crepuscular biting hours and residing within houses which limit sandfly access for both feeding and lekking opportunities (Costa *et al.*, 2005; Courtenay *et al.*, 2007; Quinnell and Dye, 1994a;b), which implies that humans are fed on opportunistically.

Following nonlinearity in preference through to its conclusion it may be expected that transmission rates to dogs and humans both vary nonlinearly with vector density. Nonlinearity in the pattern of modelled transmission rate appears, however, to be offset somewhat by the actual vector density. Additionally, the relationship between transmission rate to dogs and human appears to differ depending on the measure of vector density, such that when indoor estimates of vector density are used transmission to humans appears to follow a nonlinear trend with vector density. However, when outdoor estimates of vector density are used, nonlinearity in transmission rates appear to affect only the dogs. This may be because the density-dependent preference for one host in particular drives the nonlinearity in transmission between hosts. However, from the data investigated here it is not possible to confirm which host is more critical in driving the nonlinearity seen in prevalence data. Additionally, high variability in the average village vector density when calculated from outdoor household densities rather than indoor fly densities

may lead to slightly different patterns depending on the measure of vector density employed. In reality, an accurate measure of vector density across the peridomestic setting or upon specific hosts would improve understanding of how preference and transmission rates relate to one another, an issue to be addressed in later chapters (Chapter 3).

Despite obvious heterogeneity in transmission processes, population averaged models are frequently adopted for mathematical convenience. The data presented here challenge the basic assumption of linearity commonly adopted in published multiple-host VL models (Dye, 1996), stating that infection rates in the reservoir and human populations are linearly related, being proportional to one another as a function of static or host density-dependent biting rates. Heterogeneity in the biting rate on subpopulations of dogs (Dye *et al.*, 1992) and the infectiousness of subpopulations of dogs (Lanotte *et al.*, 1979) has, however, been previously considered within a modelling framework (Hasibeder *et al.*, 1992). Hasibeder *et al.* (1992) constructed several formulations for the estimation of R_0 of zoonotic VL in heterogeneous dog populations. However, constant biting preferences were assumed for each subpopulation, which, as demonstrated here using different species populations, does not appear to be the case. Quantification of the relationships between vector density and biting preferences may permit further extension of the R_0 formulations presented here and by Hasibeder *et al.* (1992) allowing R_0 to be calculated using just the above model and vector density estimates.

The results here indicate that insecticidal control of vector populations may be counterproductive at high vector densities as this may disrupt the preference for

chickens and result in increased preference for dogs and humans, and thus impact counterproductively upon transmission rates. What this means in terms of the impact of insecticide is unclear, but may indicate that the impact of vector density reduction on transmission may depend on the starting point of vector density. Under the linear assumption, a reduction in sandfly density of 50% would reduce R_0 by 50% regardless of the starting density (Hasibeder *et al.*, 1992). These results suggest that the impact of vector reduction on transmission will be influenced by initial sandfly density. In the most perverse situation, as decrease in sandfly density may increase the transmission rate if it results in a switch in preference from chickens to dogs. In practical terms this may serve as a warning to the use of insecticide in some situations, and highlights that inadequate vector control has the potential to be counterproductive. Future models of control would need to take any such density dependence into account.

The density-dependent process could also explain the possible negative relationship between preference for dogs and chickens. This may indicate that any increase in preference for chickens, due to effects of vector or host density, could divert flies away from dogs onto non-competent hosts. Therefore access to alternative hosts may confer some protection against infection by diluting biting and transmission rates and offers hope for the use of chickens in zooprophylaxis. This concept is at odds with guidelines dispensed by some Brazilian municipalities which advise against keeping chickens, due to their contribution towards local noise and odour (Courtenay, pers. comm.) and possible importance as a maintenance host for sandfly populations (Alexander *et al.*, 2002). However, it should be noted that the preference for chickens appears to plateau at high vector densities, and there is little data to indicate what

would happen to the transmission rate in humans and dogs if vector density continued to increase and lekking upon chickens was to saturate. It may therefore be hypothesised that in areas of low chicken availability or even higher vector density, that preference would return to susceptible hosts and again increase transmission. However, Marajó possesses some of the highest known areas of *Lutzomyia* density in the world, therefore the latter part of this hypothesis would be difficult to investigate further. There is also a risk that chicken odours may attract flies over larger distances than alternative odours resulting in high vector densities, or promoting the interaction of putative sylvatic *Leishmania* hosts (e.g. foxes predating on chickens) with domestic vector populations in rural areas, as suggested by Alexander *et al.* (2002). Additionally, introducing or removing chickens might influence local sandfly density if host availability is a limiting factor in population carrying capacity. This is not however, included within the framework of this chapter and this potential impact is difficult to predict and requires a further model. The importance of chickens within the epidemiology of VL therefore remains complex, and requires further elucidation in the field.

The data presented here show some limitations; neither the prevalence nor vector density data were collected for the specific purpose presented here and despite being collected by comparable methods, do differ slightly in their approaches and timing. It should be made clear that in both humans and dogs serological and MST positivity are indicative of exposure to *Leishmania* eliciting an immune response, and may not be indicative of current infection status (Ferrer *et al.*, 1995; Ferrer, 1999). For this reason these tests give a good indication of the biting pressure upon each host, rather than clinical infection which is dependent upon many other factors, such as

nutritional status, and host immunity. Nevertheless, human and canine prevalence data were obtained using differing serological tests, antigen preparations and elements of experimental design between investigations, which may limit the comparability of the data and thus confidence in conclusions. For example, either IFAT or ELISA was used upon dogs, these have both been shown to detect over 90% of canine leishmaniasis (Mancianti *et al.*, 1996), yet some labs have identified IFAT sensitivity as being much lower (Quinnell *et al.*, 1997). Additionally, the rate of loss of MST positivity appears higher in these Brazilian data than previously reported for Iran (Davies and Mazloumi-Gavgani, 1999). Any differences in loss of MST responsiveness may, however, be dependent upon the local human and parasite population in addition to antigen preparations. It was known that differences between antigen batches can play an important role in test sensitivity over time (Bern *et al.*, 2006). Finally, it is assumed in the model development that the transmission dynamics are at equilibrium, and have remained at equilibrium for a human lifetime (the longest compartment in the model).

Nevertheless, very few studies have attempted to sample sandfly density and infection status of both humans and dogs, therefore this represents the most comparable and comprehensive dataset possible, and explains why there are so few data points with high percentage prevalence in dogs included in this study. It should be noted that if canine prevalence were only investigated, up to ~65%, the relationship would appear linear. The nonlinear result presented here comes from the five points where canine prevalence is in excess of 80%. The absence of additional points at such as high prevalence may also reflect inadequacies and in sensitivity of some serological diagnostic methods (or antigens), such some prevalence results are

incorrectly reported, despite their apparent importance in determining the nature of the relationship between infection in susceptible populations.

Additionally, vector density estimates were only available for a subset of the surveyed villages, but were nevertheless assumed to be representative of host preference and transmission relationships underlying the nonlinearity in prevalence. Investigation over a greater number of locations and a wider range of vector densities is therefore required to confirm these relationships. Here, the indoor or total (indoor and outdoor) household geometric mean fly density for a given village was calculated, however, this does not reveal the absolute local vector density and ignores potentially important heterogeneity in density between nights and houses, which may be a source of error. Nevertheless, it was necessary for comparison with village level prevalence data. Relationships also appear to differ depending on vector density estimate used. This is potentially due to greater variation in outdoor household sandfly density estimates, which leads to less meaningful averages.

2.6. Conclusions

Overall, analysis of human and canine prevalence data and investigation of corresponding model-derived sandfly host preferences has revealed the existence of nonlinear relationships between prevalence in dogs and humans. Such nonlinearity may indicate the existence of vector density-dependent feeding preference effects. These could have important implications for predictive modelling and current understanding of vector control. However, thus far, the dynamics of feeding preference have only been demonstrated through the use of mathematical models. Greater understanding of vector behaviour with respect to host choice is necessary to

further investigate and confirm these relationships, and therefore worthy of experimental examination over a range of vector and host densities in the field.

Chapter 3: **Method development: sandfly trapping efficiency and bias**

3.1. Introduction

The dynamics of zoonotic visceral leishmaniasis transmission are critically dependent upon the infective biting rate of sandflies upon the key susceptible hosts, dogs and humans. *Lutzomyia* longipalpis are, however, eclectic in their feeding behaviour and readily feed upon a wide range of hosts (Deane and Deane, 1962; Morrison et al., 1993b). Consequently, absolute biting rates and relative preferences for dogs and humans are influenced by the attractiveness of alternative non-competent hosts, such as chickens (Campbell-Lendrum et al., 1999a) (see Chapter 2). Appreciating the dynamics of sandfly preference across multiple host types is therefore vital to improving understanding of infective biting rates, transmission and control.

Infective biting rates are determined by the number of sandfly bites which occur per host per night which may transmit infection (Keeling and Pejman, 2008). The infection status of sandflies can be directly assessed using molecular techniques (Acardi et al., 2010; Almeida Felipe et al., 2011) or microscopy (Brazil and Ryan, 1984), but this can also be complemented by estimates derived from the proportion of sandflies feeding on dogs and infected dogs (as proposed in Chapter 2). Nightly preference and biting rate per host may be appraised from the number of sandflies caught simultaneously upon different hosts. Each host species' environment, however, presents unique problems in terms of both maximising trapping efficiency and minimising bias. These must be resolved by the selection of appropriate trapping

techniques in order to reflect accurately the absolute and relative number of sandflies found concurrently upon different hosts.

There is no gold standard method for sandfly sampling; instead a large number of techniques are available which are appropriate for investigating different questions. These range from attraction-based techniques, such as light traps, to interception-based techniques, such as flight traps, all of which can be augmented by the addition of hosts to potentially assess preference. Different techniques, however, sample from different portions of the sandfly population, as behavioural changes during the sandfly life-cycle and gonotrophic cycle ensure sandflies are not at equal risk of capture at all times or life stages. For example, host seeking females predominate in animal baited traps, while gravid females at rest or in search of oviposition sites are comparatively unresponsive to host stimuli and are underrepresented by this trapping method (Alexander, 2000).

The most common method for sandfly capture is the standard miniature Centre for Disease Control light trap (referred to henceforth as CDCLT) (Alexander and Maroli, 2003; Faiman *et al.*, 2009). Using a light source this trap exploits the phototactic response of nocturnal insects such as sandflies, to encourage an encounter with the mouth of a suction-trap (see Figure 3.3). CDCLT are a non-labour-intensive means of sandfly capture, capable of catching large numbers of nocturnal insects in a single night (Muirhead-Thompson, 1991). The light in CDCLT is particularly effective at attracting sandflies over distance; increasing the sample area, actively recruiting flies into the trap. This effectiveness over distance has the benefit of reducing problems of positional bias (Davies *et al.*, 1995). For example Alexander and Young (1992)

placed CDCLT at different heights within a coffee plantation and caught similar sandfly numbers in all traps, which indicates that phototactic responses may overcome differences in vertical distribution in some species. Responsiveness to light over distance is, however, highly variable between species, ranging from 6m in *Lu. youngi* (Valenta *et al.*, 1995) to less than 2m in *P. ariasi* (Killick-Kendrick *et al.*, 1985). Consequently, CDCLT often provide a strongly species biased sample (Chaniotis *et al.*, 1971). Within a species however, CDCLT captures remain relatively unbiased according to fly sex and physiological state (Alexander and Young, 1992; Gibb *et al.*, 1988), but as host-seeking females are highly active they can be preferentially captured over males (Alexander, 2000), whereas males that promote female aggregation e.g. by pheromone may be preferentially captured, depending on factors such as the timing of capture (Dye *et al.*, 1991; Kelly *et al.*, 1997).

CDCLT, are often used in conjunction with host bait in order to maximise capture. All capture techniques can be augmented by the addition of a host or host odours, such as carbon dioxide, 1-octan-3-ol, lactic acid, caproic acid, ammonia or ketones detectable in exhaled breath, as these can act as long-range attractants to improve efficiency and permit estimation of host preference (Andrade *et al.*, 2008; Gibson and Torr, 1999). In the case of host baited CDCLT, however, it may be difficult to disentangle the effects of the multiple attractants. In particular, the light source may lead to over representation of the number of flies per host. Differences in visibility of the light source and proximity to host and oviposition sites may be a significant source of bias when comparing trap catches across multiple domestic locations. For example, variability in the ratio of parous: non-parous females between traps may be

due to differences in proximity to host and oviposition sites (Alexander and Young, 1992), which are minimised but not eradicated by the use of light stimuli.

Alternatively, interception style traps (e.g. castor oil "sticky" traps), placed across the flight paths of sandflies, can be used to sample flies without the bias of light attractants, leading to capture of active flies of all species, sex and stages of parity (Ferro *et al.*, 1995b). For this reason they are commonly used to describe local species compositions (Ferro *et al.*, 1995b). An interception trap is any trap without an attractant; sticky traps, which capture flies when they alight upon castor oil-coated surfaces (Molyneux and Ashford, 1983), or netting which intercepts flies during flight and funnels them into a kill jar, such as the Malaise trap (Gressitt and Gressitt, 1962), are most commonly used. Sticky traps are cheap and easy to manufacture and can therefore be used to sample over large areas. Yet capture rate with interception traps is dependent upon the number of flies active within the immediate trap vicinity, leading to typically low capture rates, and problems with positional bias whereby good trap placement, such as proximity to host or flight route, is vitally important to capture rate. For example Faiman *et al.* (2009) demonstrated, in the absence of light, that CDCLT positioning strongly influences capture rate. Specifically, increased proximity to the ground by the inversion of CO_2 baited CDCLT without light can improve capture rate among species such as *P. papatasi* and *P. sergenti* (Faiman et al., 2009). This is because it maximises on both the negative geotropic response of these sandflies to obstacles including changes of air flow (Muirhead-Thompson, 1991), but also because it ensures the mouth of the trap is in close association with the ground, where sandflies may travel by their typically hopping flight, and close to CO_2 gradients, which may be along the ground for heavy gasses such as CO_2

(Faiman *et al.*, 2009). Alternatively, the use of interception style traps in abundance can reduce issues of positional bias.

Sticky traps can be modified by the addition of hosts too, for example a caged host can be placed in the centre of a large sticky trap (Disney trap) in order to capture flies as they visit and leave hosts, which they normally do via a series of short hopping flights (Disney, 1966). Disney traps may however prove difficult to standardise across hosts sleeping in different environments, and thus make comparison difficult. Capture on sticky traps may also lead to insect damage making sandfly identification and separation by sex difficult (Alexander, 2000). Traditionally, many interception trap types use killing agents in the collecting chambers so as to better preserve insect specimens; this may reduce sandfly capture rate through the effective removal of pheromone production important to recruitment, or possible repellent effects due to insecticide or volatile killing agents (Kelly & Dye, 1997), leading to under representation on some hosts.

Direct aspiration of sandflies from hosts or light attractants, such as in the Shannon traps or carrying out humans landing catches (HLC), represent another way to estimate the number of sandflies attracted to the stimulus. Direct aspiration from animal hosts has been reported as an efficient means of *Lu. longipalpis* capture, compared to sticky and Disney traps (Ferro *et al.*, 1995b), and HLC is a standard method to estimate flies potentially biting humans in different locations (e.g. indoors *vs* outdoors)(Courtenay *et al.*, 2007). Yet, these methods are all highly intensive, and therefore difficult to employ on a wide scale. Additionally, differences in collector ability and the relative ease of capture upon different hosts and in different

microhabitats present a significant challenge to aspiration feasibility and catch comparability between locations (Alexander, 2000), plus, the presence of human collectors may confound sandfly preferences. Innovative improvements upon direct aspiration are available. The use of cone traps whereby an animal is placed within a large net with two funnels at either end (Montoya-Lerma and Lane, 1996) or box traps upon humans sleeping inside under an additional layer of netting (Maroli *et al.*, 1997), allow flies to enter but not exit, so they can be aspirated at the end of the trapping period. Such traps permit the diffusion of host odours and remove the need for human collectors throughout the trapping period. However, restricting fly entry points will negatively affect trap entry as many sandflies will be diverted away by the outer surface. This inefficiency may be further compounded by reductions in sex pheromone production, as fewer males are able to enter and commence lekking leading to under representation and bias in samples by these methods.

As an alternative to host odour, other attractants such as the sandfly sex pheromone, which play a role in sandfly recruitment (Kelly and Dye, 1997), may be used to bait traps. Tests in the field have revealed that pheromone can act both as an attractant (Morton and Ward, 1990) or even repellent if applied in too high a concentration (Morton and Ward, 1989; Ward *et al.*, 1990). Nevertheless, simulated sex pheromone production will affect long-range recruitment and potentially confound relationships between hosts and sandfly preference in which sandfly pheromone production may play an important part, and is therefore unsuitable for assessing host choice.

Overall, there are challenges common to the use of all the above techniques in the estimation of biting preference, for the reasons discussed. Animal ownership

practices in the New World typically involve dogs sleeping outdoors, while chickens are housed within a chicken shed of spaced wooden stick and palm leaf construction. Therefore all hosts of interest are differently enclosed so that the factors important to sandfly trapping, namely trap accessibility, light visibility and, exposure to elements, differ across localities. Furthermore, by virtue of being in an enclosed area, flies in sheds and houses may be more likely to encounter a trap than those free to visit an unenclosed dog, which may contribute towards a bias in trapping success across these locations (Quinnell *et al.*, 1992). Quinnell and Dye (1994b) demonstrated that the volume of the area from which flies were being sampled was, however, not statistically significant compared to the disparity in recruitment rates to different host locations, when houses and chicken sheds were compared. This effect of effective sample volume could, however, be more marked upon dogs and is a potential source of bias. Finally, additional factors such as distance between hosts and proximity to resting sites and external attractants such as lights may differently influence capture upon all hosts (Muirhead-Thompson, 1991; Williams, 1936).

3.2. Aims

The principal aim of this Chapter is to assess the trapping success, and estimate the bias associated with each trap type, by comparing four different trapping methods. Specifically, the aim is to explore methods which reduce trapping biases across three key peridomestic hosts while maximising yield, thus to provide data which reflect preference, whilst maximising sample size and statistical power. In order to do this in the field it was necessary to develop a new technique for trapping sandflies on dogs (for reasons given above), and address the following objectives:

1. Compare four different methods of trapping sandflies upon dogs to determine which yield the greatest sandfly numbers.
2. Compare four different methods of trapping sandflies upon humans to determine which yield the greatest sandfly numbers.
3. Compare four different methods of trapping sandflies upon chickens to determine which yield the greatest sandfly numbers.
4. Investigate the relationship between the numbers of sandflies caught at each trapping location by CDCLT methods in order to assess bias associated with light.
5. Compare capture rates of sandflies at three periods during the night with respect to trapping efficiency in order to optimise capture period and duration.
6. Investigate the relationship between the number of sandflies caught by human landing catch and CDCLT upon humans to determine if CDCLT are appropriate for estimating the number of sandflies visiting humans.

3.3. Methodology

3.3.1. Study houses

The study took place in the villages of Bacabal (BA) and Boa Vista (BV) in the Salvaterra district of Marajó Island, Pará State, in northern Brazil (Figure 3.1). These villages were selected due to previously reported high vector density and human case incidence (MoH records; Roberto Bahia pers. comm.; see Chapter 2) and their relative proximity to Salvaterra, facilitating their use as preliminary study sites. The main trap comparison experiment took place during the dry season from late September to the end of October 2011 in BA, and additional investigations of human

landing catches and sandfly activity took place from December 2011 and January 2012 in BV, where intermittent rains began in January.

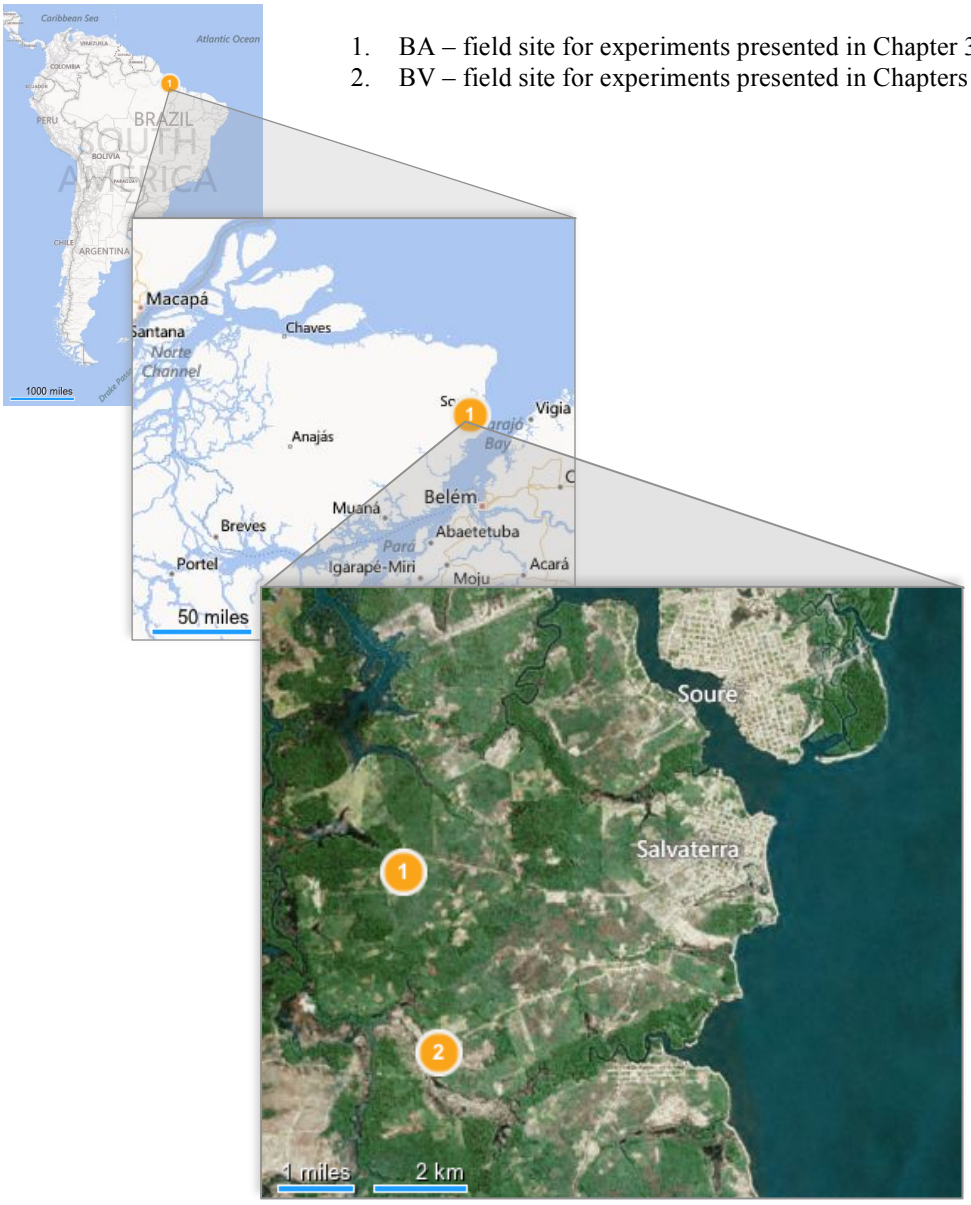


Figure 3.1: Map of South America with cascading inset map of the alluvial island of Marajó and aerial photograph of the field location of the two study sites on the island. Images adapted from Bing Maps and aerial imagery (Microsoft Corporation 2013, Earthstar Geographics SIO, Nokia 2013).

Rural houses in these areas are typically of a traditional adobe construction of clay-lime plastered onto a wooden frame with thatched roofing (Quinnell and Dye, 1994a) (see Figure 3.2a). However, half (two) of the study houses in BA and six of the eight

study houses in BV were built of brick with a tile roof (Figure 3.2a). One household in BV was of wooden plank construction (Figure 3.2b).

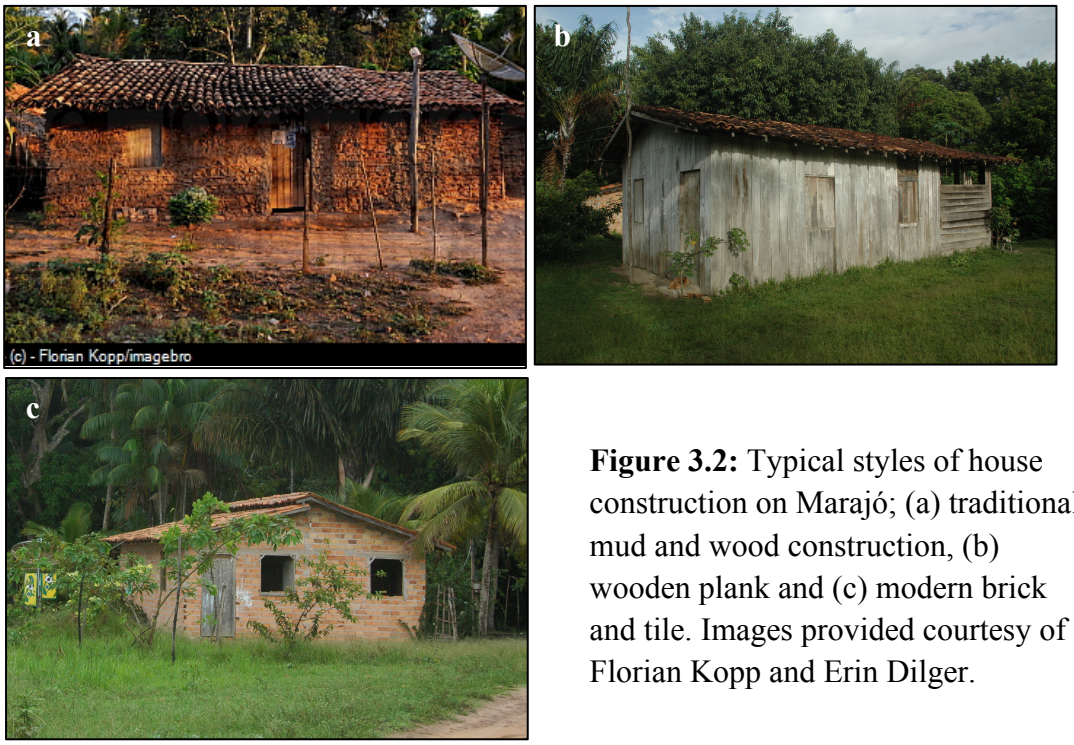


Figure 3.2: Typical styles of house construction on Marajó; (a) traditional mud and wood construction, (b) wooden plank and (c) modern brick and tile. Images provided courtesy of Florian Kopp and Erin Dilger.

In the village of BA, and following initial night-time trapping at seven consenting households which had a resident human, dog and chicken, four households were selected which had the highest sandfly density (summed across the three domestic trapping sites (V_S)) and which met strict selection criteria for inclusion in the trap comparison study. The inclusion criteria were that the household must be home to humans, an adult dog and chickens which all sleep on site, with chickens roosting within a chicken shed. Throughout the study period these four households were home to an average of 4.9 people (range 3-9), 4.4 dogs (range 1-10), 15.2 chickens (range 10-20) and 2.3 other hosts (range 1-5) comprising of ducks, cats and pet birds, and in one household, a buffalo.

In BV, an initial three nights trapping was carried out in 12 consenting households which met the above selection criteria. Of these the eight with the highest geometric

mean density of flies were selected as locations for investigation of HLC trap efficacy and timing of sandfly activity.

3.3.2. Collection protocol

On trapping nights, traps were set in three domestic locations in close association with each host of interest; one trap in the bedroom of the house, one in the chicken shed and one trap in association with a dog. To standardise trapping, dogs were caged on trapping nights, and the same dog placed in the cage when possible in households with multiple dogs. If the main dog was unavailable an alternative animal was used the identity of which was recorded in order to take this into account in the analysis. All traps were then positioned as close as possible to the head height of each respective host. Traps were placed equidistant from each other, i.e. caged dogs were placed under the shelter of a tree at a distance from the house and chicken shed equal to the distance between these two permanent structures. This was aimed at minimise the influence of distance between hosts on sandfly host preferences, and to ensure dogs were not in close proximity to potential confounders such as lights, cooking facilities or other hosts.

Traps were set at 6.30pm and collected 6.00am the following morning. Upon collection, flies from each catch were transferred to ethanol. Sandflies were identified by external morphology and separated by sex before being counted under a dissection microscope. Identification of sandflies to the species level was not carried out as the vast majority of sandflies in this area have been previously identified, on repeated occasions, as *Lu. longipalpis*; *Lu. longipalpis* comprise over 93% of sandfly

catches in this area across all peridomestic locations (Kelly *et al.*, 1997; Lainson *et al.*, 1990), as identified by examination of spermatheca and pharyngeal armature described by Ryan (1986).

3.3.3. Trap comparison and optimisation on dogs

To optimise the capture of sandflies upon dogs and compare sandfly numbers at each domestic trapping location the following four methods of capture were compared: (i) standard CDCLT with the light on (CDC_{ON}), (ii) CDCLT with no bulb (CDC_{OFF}), (iii) modified CDCLT upon dogs with light on (CDC_{MOD}) (Figure 3.3) and (iv) direct aspiration.

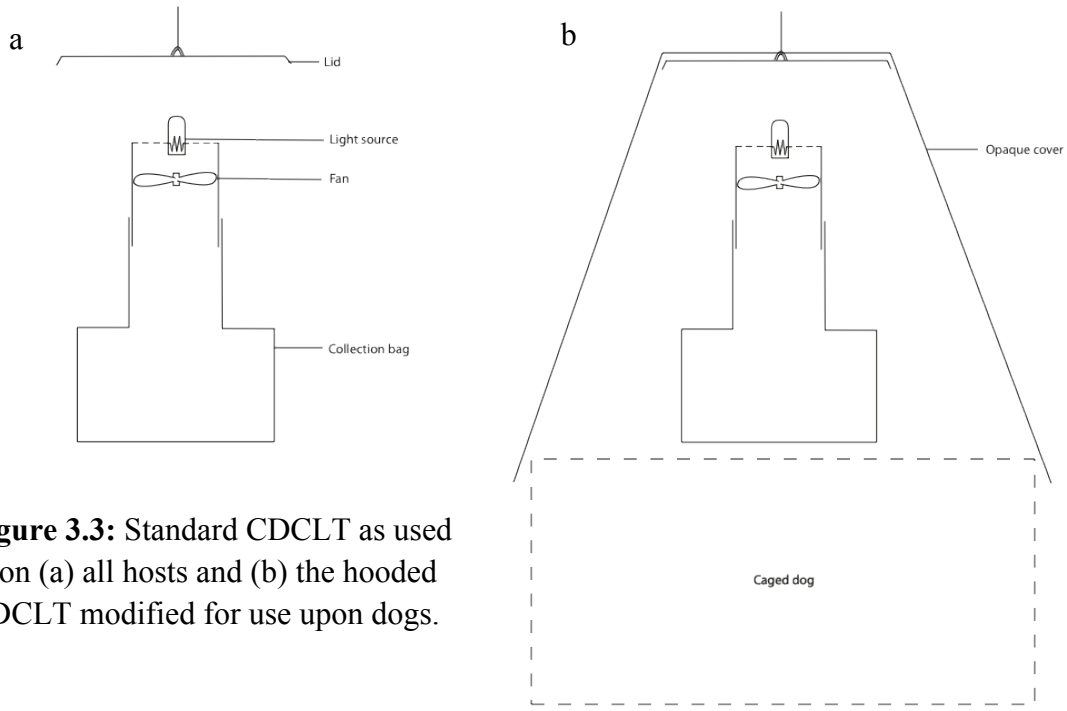


Figure 3.3: Standard CDCLT as used upon (a) all hosts and (b) the hooded CDCLT modified for use upon dogs.

CDCLT without bulb are effectively an interception trap and were used to catch only the active flies within the vicinity of the host and thus assess the relative number of flies on each host without the added attraction and potential confounder of light stimulus.

Modified traps were used to try and improve the specificity of sandfly capture on dogs, by minimising the capture of flies attracted by the presence of light alone, but maximising the capture rate of flies visiting caged dogs. To achieve this a standard CDCLT was enclosed within an opaque pyramidal hood set above a caged dog, to shield the light stimulus (Figure 3.3(b)) and to help funnel flies attracted to the dog into the trap directly above, exploiting their photo-and geotactic responses. This modification mirrors the way in which sandfly trapping may be influenced by the presence of roofs and walls in chicken sheds and houses. Hence, on modified trapping nights (CDC_{MOD}) modified traps were placed on dogs only and standard CDCLT were placed at humans and chickens.

Aspiration was used to provide a direct mechanical means of fly capture for comparison with fly numbers caught by the alternative capture methods. To carry out aspiration trained fieldworkers aspirated sandflies from hosts and the walls of their dwellings for two 20-minute intervals per night during peak biting hours (7.00pm-10.00pm (Courtenay *et al.*, 2007)), rotating between the 3 domestic locations in order (chicken shed, house then dog). Twenty-minute intervals were selected to minimise the effect of investigator presence and ensure that each host type received equal trapping effort. Two fieldworkers, working alternately, in two separate houses, carried this out. A pair of houses was sampled each aspiration trapping night until each of the four selected households had been fully sampled once by each fieldworker.

To avoid local sandfly depletion a trapping regime was adopted whereby the potentially population depleting CDCLT captures were not carried out on consecutive nights and there were three nights of zero capture per week. Therefore for every seven days, there were four trapping nights, over which the four trapping methods were rotated on a nightly basis.

Night 1: CDC_{ON}

Night 2: CDC_{OFF}

Night 3: CDC_{MOD}

Night 4: Direct aspiration

Five replicates were carried out over five weeks, resulting in 20 nights of data for each CDCLT trapping method and eight nights of aspiration capture data.

3.3.4. Trap optimisation upon humans

In addition to the main trap comparison, human landing catches (HLC) were performed concurrently with CDCLT capture within eight BV households in order to measure the effectiveness of standard CDCLT captures in assessing biting pressure upon humans.

To carry out the HLC four operators worked shirtless and in pairs, and aspirated landed flies off one another and in their immediate vicinity for a period of 2.5 hours between 6.30-9.00pm or 9.30pm-midnight, the period of sandfly activity (Courtenay *et al.*, 2007). Standard CDCLT capture was carried out simultaneously and for the

same duration in the main bedroom of the house. HLC were carried out in the doorway of each house to approximate the inside and outside nature of human activity during these hours. This protocol permitted the survey of 2 households by each HLC pair per night, including a 30-minute rest at the changeover to prevent fatigue. HLC pairs alternated between households and start time worked in each household in order to avoid aspirator bias.

Initially, the experiment was conducted in four households for one month, and was later extended to include a further four houses in the trapping regime on a regular basis, as dictated by the experimental design of primary experiments (see Chapter 4). This culminated in a total of 134 nights of trapping data across eight houses.

During this time CDCLT with the light on were set at the chicken shed, and modified CDCLT set at the caged dog.

3.3.5. Night-time sandfly activity

In order to optimise the night-time period of trapping, sandfly activity over the course of the night was monitored in a total of four consenting households in BV (described above in section 3.3.4).

CDCLT with the light on were set inside the house and chicken shed, and modified CDCLT set at the caged dog. Sandfly collection bags were then changed at regular intervals through the night so that the number of sandflies caught on each host type could be counted for three separate time periods. Collections were carried out from

6.30-9.15pm and 9.15pm-midnight in four houses for a period of one month. This was later extended to include a further four households and a third trapping period from midnight-6.30am was also sampled. At changeover, used collection bags were closed but left hanging with the traps, so as not to disrupt vector-dependent recruitment to hosts. The number of sandflies captured upon all three hosts was summed together in order to assess total capture rate at each period.

3.3.6. Survey

Information was also gathered on potential confounders such as host densities and household and environmental factors such as insecticide use and weather, which may contribute towards variation in sandfly capture rates between houses and nights (see Table 3.1 for a full list). In particular, the number of hosts often differed between the trap location and the overall household, such as the number of people. Therefore data on the number of relevant hosts sleeping at and away from each trap location were also collected. Two BA households were home to multiple dogs; therefore the number of dogs away from the caged dog and the identity of the dog at the trap were noted. A total of two and three different dogs were each used as bait for one night or more in these households and their identity recoded as potentially important confounders.

Insecticidal spraying houses against leishmaniasis and fogging against dengue fever vectors respectively is occasionally carried out on Marajó by local health authorities, yet consultation with householders and local health authorities confirmed that the last

round of insecticide application in these villages took place over 2 years prior to the start of this study.

3.3.7. Ethical approval

Informed consent was obtained for all work undertaken. All HLC were carried out by trained local operators known to have previous exposure to leishmaniasis (MST positive), in accordance with ethical guidelines.

Ethical guidance was given by the local research institution, Fiocruz, and applies to this and all subsequent experiments.

3.3.8. Analysis

All analyses were carried out using STATA v11.0 (StataCorp LP).

The number of flies caught by different trap types was log transformed [$ln(n+1)$] to approximate a normal distribution for parametric analysis (see Appendix D). Fly number by trap was compared for each host using linear mixed effect models via the *xtmixed* command in STATA. In order to aid the interpretation of comparative model outcomes, the exponent of the log-average values [$exp(n)-1$] was taken to give the geometic mean number of sandflies per trap type (Williams, 1937). Household ID and trap night were included as cross-classified random effects within these models (Fielding and Goldstein, 2006), to take into account unobserved heterogeneity over each house and trap night. The number of sandflies per trap was the outcome under

investigation, and trap type the key predictor of interest. CDC_{OFF} was the interception style trap, and was therefore assumed to be the least biased trapping method and used as the reference category throughout. Covariate predictors of sandfly density were then tested in this basic model as fixed effects (Table 3.1). Significant predictors were then used as possible variables for inclusion in a full model, developed using a forward stepwise approach.

If there was equipment failure, heavy rain or absence of the trap specific host, for example, if the dog escaped during the night's capture, these nights were excluded from the host specific analysis.

Table 3.1: List of fixed effect covariates.

Household Factors	*Host Densities /per house and per trap*	*Environmental Factors*
Wall type	Adults	Rain during day y/n
Roof type	Children	Rain on capture night y/n
Stilts y/n	Total humans	Wind (scale of 1-4)
Age of house	Dogs	Vegetation type
Light y/n	Large chickens	Refuse burning y/n
Outside light source y/n	Small chickens	
TV outside y/n	Total chickens	
Time outside lights extinguished	Pigs	
House openness	Buffalo	
Bednets y/n	Horse	
Insecticide/repellent y/n	Ducks	
ITN y/n	Others	
IRS house y/n	Total hosts	
IRS chicken shed y/n	Total alternative hosts	
Chicken shed openness		
Roost height		
Dog ID at trap		
Distance between hosts of interest		

HLC data were highly over dispersed and therefore analyses were carried out using negative binomial regression, while continuing to taking into account structuring in the data due to clustering upon household via their inclusion as random effects. Night was not included as a random effect in this analysis because CDCLT and HLC capture were carried out simultaneously.

To compare capture rates over different periods of the night the logged total captures for each trapping period [*ln(n+1)*] were compared, taking into account clustering within the data on household ID. This was again performed using a linear mixed model, incorporating house ID as a random effect. The number of flies caught in the first trapping period (6.30pm-915pm), was used as the reference category.

3.3.9. Trap bias analysis

The relationship between different trap types was also investigated via the simple linear regression of the geometric mean number of flies caught (V_{CDC}) by two trap types. The relative efficiency (*e*) of one trap compared to another can be estimated from the regression coefficient, and the additional baseline attraction of flies to one trap compared to another (due to trapping bias) (*c*) can be found from the intercept (see equation 3.1),

$$V_{CDC_X} = e\, V_{CDC_{OFF}} + c \qquad (3.1)$$

where CDC_X is either CDC_{MOD} or CDC_{ON}.

3.4. Results

3.4.1. Data summary

To test the efficiency and bias of sandfly capture methods upon key domestic hosts, four different trap types were tested in the field. Following the application of exclusion criteria, this culminated in a total of 57 nights of sandfly collection: 16 nights by CDC_{OFF}, 17 nights by CDC_{ON}, 16 nights by CDC_{MOD} and 8 nights by manual aspiration (see Table 3.2 for full breakdown).

A total of 44,341 sandflies were caught and separated over the course of the investigation. Fly densities per trap varied widely from 0 to 7,256 flies, with the majority of flies caught on dogs and chickens, and relatively few being caught within houses (geometric mean fly density of 57.09, 49.84 and 2.16 upon dogs, chickens and humans respectively per night).

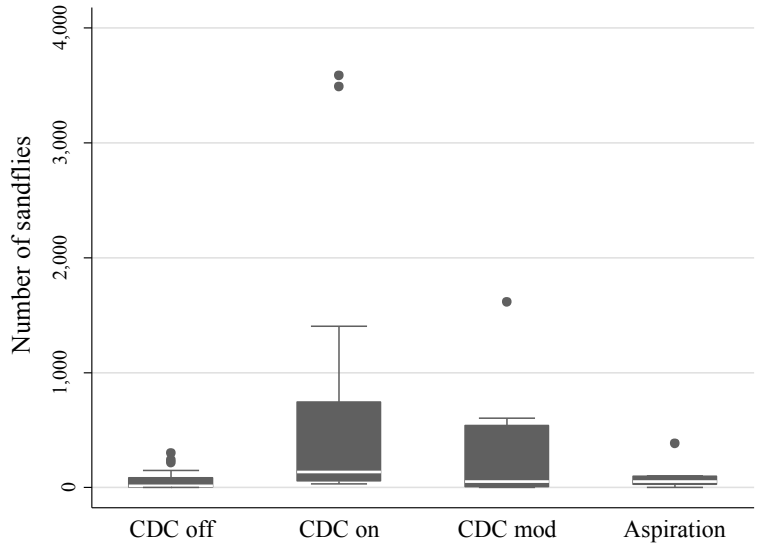
Table 3.2: Number of successful trapping nights upon each individual host type and all three hosts using four trapping methods. Trapping nights were considered unsuccessful and excluded if equipment failed or the respective host was not present.

Trap type	*Number of successful trapping nights*			
	Humans	Dogs	Chicken	All
CDC_{OFF}	19	17	18	16
CDC_{ON}	18	18	17	17
CDC_{MOD}	19	16	19	16
Aspiration	8	8	8	8

3.4.2. Comparison of trap yield: Dogs

Upon dogs, CDC_{ON} traps caught the greatest number of sandflies, followed by CDC_{MOD}, aspiration and CDC_{OFF} (Figure 3.4).

Figure 3.4: Average number of sandflies captured on dogs per night using four different trap types. White lines represent median fly densities. Whiskers and box limits represent the 95th percentiles and interquartile ranges respectively.



Structuring in the data, due to repeated measures on households and comparison of trap yield on different nights, was accounted for via the inclusion of random effects. Univariate analysis of the log-transformed data confirms that sandfly numbers caught on dogs differ significantly with trap type ($P<0.0001$). Specifically, CDC_{ON} ($\beta=2.52$, $z=5.09$, $P<0.0001$), followed by aspiration ($\beta=1.17$, $z=2.02$, $P=0.044$) and then CDC_{MOD} ($\beta=1.12$, $z=2.24$, $P=0.025$) caught statistically significantly more sandflies than CDC_{OFF} (summarised in Table 3.3).

Table 3.3: Geometric mean numbers of sandflies caught on each host by differing trap types. Geometric means are adjusted for random effects of household ID and trap night but no other predictors in the univariate model; additional adjustment by host specific significant covariate predictors was included in the multivariate model.

Model	Trap type	*Dogs* Geometric mean (S.E.)		*Humans* Geometric mean (S.E.)		*Chickens* Geometric mean (S.E.)	
Univariate	CDC_{OFF}	13.36	(1.42)	0.73	(0.49)	13.69	(1.00)
	CDC_{ON}	177.36	(1.42)	3.07	(0.49)	164.22	(1.02)
	CDC_{MOD}	43.18	(1.43)	4.66	(0.49)	128.23	(1.00)
	Aspiration	45.61	(1.54)	0.45	(0.59)	7.85	(1.21)
Multivariate	CDC_{OFF}	12.49	(0.83)	0.68	(0.29)	13.67	(0.54)
	CDC_{ON}	187.41	(0.78)	3.12	(0.30)	162.31	(0.56)
	CDC_{MOD}	46.46	(0.86)	4.65	(0.29)	126.74	(0.53)
	Aspiration	22.76	(0.76)	0.80	(0.42)	7.94	(0.79)

Covariate predictors of sandfly density were then individually incorporated into the above mixed effects model with trap type. Of these, eight were found to be statistically significant *($P \leq 0.05$)* and three of borderline significance *($P \leq 0.10$)* (Table 3.4).

The inclusion of these covariates had little effect upon the predictor of interest, trap type, except wind strength which appears to confound the relationship between trap types and the number of flies caught on dogs. When the effect of wind is taken into account the number of flies caught by CDC_{ON} (*$\beta=2.63$, $z=6.63$, $P<0.0001$)* and CDC_{MOD} (*$\beta=1.26$, $z=3.14$, $P=0.002$*) remains statistically higher than CDC_{OFF}; however aspiration (*$\beta=0.56$, $z=1.00$, NS*) becomes associated with fewer flies than CDC_{MOD} and is no longer significantly different to CDC_{OFF}.

Table 3.4: Significant predictors of sandfly density upon dogs, when included as single covariates with trap type in the fixed portion of the mixed effects model.

Variable	*Categories*	*Co-efficient (95% CI)*		*SE*	*z*	*P>z*
No. of dogs away from the trap		0.35	(0.03, 0.66)	0.16	2.13	0.030
No. dogs		0.30	(-0.03, 0.63)	0.17	1.76	0.079
No. ducks		1.00	(-0.14, 2.14)	0.58	1.71	0.087
Average distance between hosts		0.17	(0.13, 0.20)	0.02	9.94	<0.001
Bednet Y/N	No					
	Yes	-3.09	(-4.57, -1.61)	0.76	-4.09	<0.001
Wall type	Mud					
	Brick	-3.09	(-4.57, -1.61)	0.76	-4.09	<0.001
Wind strength	Still					
	Light breeze	-0.46	(-1.37, 0.46)	0.47	-0.98	0.327
	Strong breeze	-1.05	(-2.16, 0.06)	0.57	-1.85	0.065
	Strong wind	-1.27	(-2.22, -0.32)	0.48	-2.63	0.009
Rain in day	No					
Y/N	Yes	-0.86	(-1.77, 0.06)	0.47	-1.84	0.066
Dog ID	1					
	2	-0.60	(-3.04, 1.84)	1.24	-0.48	0.630
	3	-4.36	(-7.96, -0.77)	1.84	-2.38	0.017
	4	-3.67	(-6.9, -0.44)	1.65	-2.23	0.026
	5	-4.47	(-7.82, -1.12)	1.71	-2.61	0.009
	6	-2.91	(-6.12, 0.30)	1.64	-1.78	0.076
	7	-4.11	(-7.37, -0.85)	1.66	-2.47	0.013
Local vegetation	Trees					
	Manihot esculenta	3.09	(1.61, 4.57)	0.76	4.09	<0.001

Note: all variables tested individually in a model as a fixed effect along with trap type, with house ID and trap night are random effects. All presented variables are significant (P≤0.05) or borderline significant (P≤0.1).

Of the household-level factors, the use of brick in house construction and presence of a bednet are strongly negatively associated with fly density upon dogs, and local vegetation type (cassava plant (*Manihot esculenta*)) is strongly positively associated with fly density. These variables are, however, highly collinear with one another, as are all predictors which are fixed at the household level (see Appendix F). Due to this collinearity only one of the significant collinear household level predictors is

carried forward into the multivariate model, vegetation. Of the other collinear variables several may be important predictors of local fly density, but as only four houses were under observation these data are insufficient to draw conclusions on the individual effects of these covariates.

Forward stepwise introduction of the significant covariates results in the following significant parsimonious model, whereby only vegetation and wind strength remain in the model as significant predictors (Table 3.5). Fly density appears to decrease with wind strength and be higher in households surrounded by *M. esculenta* cultivation. Taking these covariates into account, CDC_{ON} is then associated with the highest fly densities followed by CDC_{MOD}, aspiration and then CDC_{OFF} (summarised in Table 3.3), although only CDC_{ON} and CDC_{MOD} catch significantly more flies than CDC_{OFF} (Table 3.5). In the final model the random effects of house and trap night become non-significant, potentially due to collinearity with fixed effects. However, random effects were forced into the model, as they account for important data structuring inherent to the experimental design.

Table 3.5: Full mixed effects model of log fly density upon dogs, taking into account data structuring.

Fixed effects	*Category*	*Co-efficient (95% CI)*		*SE*	*z*	*P>z*
Trap type	CDC_{OFF}					
	CDC_{ON}	2.64	(1.86, 3.42)	0.40	6.64	<0.001
	CDC_{MOD}	1.26	(0.47, 2.05)	0.40	3.13	0.002
	Aspiration	0.57	(-0.53, 1.67)	0.56	1.01	0.313
Vegetation	Trees					
	M. esculenta	3.16	(1.53, 4.78)	0.83	3.81	<0.001
Wind strength	Still					
	Light breeze	-0.46	(-1.38, 0.45)	0.47	-0.99	0.324
	Strong breeze	-1.01	(-2.12, 0.1)	0.56	-1.79	0.073
	Strong wind	-1.26	(-2.21, -0.32)	0.48	-2.62	0.009
Intercept		2.60	(1.42, 3.79)	0.60	4.31	<0.001

Random effects	Estimate	(95% CI)	SE
House ID	0.66	(0.20, 2.15)	0.40
Trap night	3.80E-08	(3.28E-13, 4.39E-3)	2.26E-07

3.4.3. Comparison of trap yield: Humans

Sandfly densities appear very low in all houses by all capture methods (Table 3.3). The highest capture densities are by CDCLT with the light on, which refers to both CDC_{ON} and CDC_{MOD} trapping nights, where the trap type on humans was in fact the same (Figure 3.5).

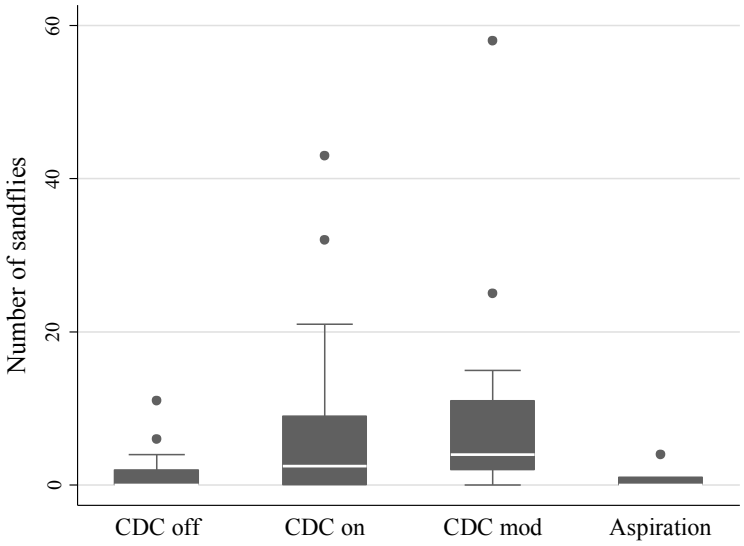


Figure 3.5: Average number of sandflies found upon humans by four different trap types. Median fly numbers are represented by white lines. Whiskers and box limits represent the 95th percentiles and interquartile ranges respectively.

Univariate analysis shows that the difference between the number of flies caught by aspiration and CDC_{OFF} is non-significant (*β=1.17, z=2.57, NS*) and the number of flies caught by either method is very low (Table 3.3). CDC_{MOD} (*β=1.19, z=3.26, P=0.001*) and CDC_{ON} (*β=0.86, z=2.33, P=0.020*) caught statistically significantly more flies than CDC_{OFF}. However, despite the same trap equipment being used within houses on CDC_{ON} and CDC_{MOD} trapping nights, there appears to be on average 1.59 flies more per CDC_{MOD} trapping night than CDC_{ON} nights, although the difference does not appear to be significant when CDC_{MOD} is used as the reference category (*β=-0.29, z=-0.80, P=NS*).

Out of 37 possible covariate predictors, individual inclusion of each into the mixed effects model shows that eight are significantly associated and two are borderline associated with sandfly density upon humans (Table 3.6). In contrast to capture rate

on dogs, none of the covariates appear to be confounded with trap type, and wind has no significant effect upon fly density.

All the covariates, however, display strong collinearity with one another (see Appendix F). This is again due to consistent differences between study houses or, as is the case with the number of humans and adults at the trap, because these variables are inherently related. As previously discussed, the most significant collinear variable is used for further model development. Following a forward stepwise approach the following significant model is reached (Table 3.7) which, similar to the initial univariate analysis of trap type demonstrates that CDC_{MOD} followed by CDC_{ON} catch the greatest fly numbers inside houses (Table 3.3). As was the case with fly density upon dogs the random effect portion of the model becomes non-significant with the inclusion of significant household-level predictors.

Table 3.6: Significant predictors of sandfly density upon humans, when included as single covariates with trap type in the fixed portion of the mixed effects model.

Variable	*Categories*	*Co-efficient*	*(95% CI)*	*SE*	*z*	*P>z*
No. adults at the trap		-0.54	(-1.01, -0.07)	0.24	-2.23	0.026
No. humans at the trap		-0.43	(-0.84, -0.02)	0.21	-2.03	0.042
No. adults		-0.42	(-0.92, 0.08)	0.25	-1.66	0.096
No. large chickens		0.12	(0.00, 0.24)	0.06	2.04	0.041
No. ducks		0.73	(0.22, 1.24)	0.26	2.80	0.005
Average distance between traps		0.06	(0.04, 0.09)	0.01	5.29	<0.001
Wall type	Mud					
	Brick	-1.16	(-1.86, -0.45)	0.36	-3.21	0.001
Bednet Y/N	No					
	Yes	-1.16	(-1.86, -0.45)	0.36	-3.21	0.001
Local vegetation	Trees					
	Manioc	1.16	(0.45, 1.86)	0.36	3.21	0.001

Note: all variables tested individually in a model as a fixed effect along with trap type, with house ID and trap night are random effects. All presented variables are significant ($P \leq 0.05$) or borderline significant ($P \leq 0.1$).

Table 3.7: Full mixed effects model of log fly density upon humans, taking into account data structuring.

Fixed effects	*Category*	*Co-efficient (95% CI)*	*SE*	*z*	*P>z*
Trap type	CDC_{OFF}	- -	-	-	-
	CDC_{ON}	0.90 (0.19, 1.61)	0.36	2.47	0.013
	CDC_{MOD}	1.21 (0.51, 1.92)	0.36	3.37	0.001
	Aspiration	0.07 (-0.79, 0.93)	0.44	0.16	0.875
Av. distance between hosts		0.05 (0.03, 0.08)	0.01	4.23	<0.001
No. adults at the trap		-0.43 (-0.82, -0.04)	0.20	-2.17	0.030
Intercept		0.26 (-0.66, 1.17)	0.46	0.55	0.583

Random effects	Estimate	(95% CI)	SE
House ID	3.17E-09	(0, .)	1.04E-05
Trap night	0.40	(0.19, 0.87)	0.16

3.4.4. Comparison of trap yield: Chickens

Capture of sandflies on chickens accounts for much of the sandfly activity. However, there is much variability in the number of flies caught upon chickens (0-7,525 flies by CDC_{MOD}). According to both median (Figure 3.6) and geometric measures (Table 3.3) of central tendency CDC_{ON} (β*=2.42, z=3.98, P≤0.001*) followed by CDC_{MOD} (β*=2.17, z=3.66, P≤0.0001*) are associated with significantly greater catches than CDC_{OFF}. However the difference between CDC_{ON} and CDC_{MOD} remains non-significant, as shown when CDC_{MOD} is used as the reference category (β*=0.24, z=0.41, P=NS*). Aspiration appears to be highly inefficient, catching fewer flies than CDC_{OFF} (Table 3.3), though the difference is non-significant (β*=-0.51, z=-0.71, NS*).

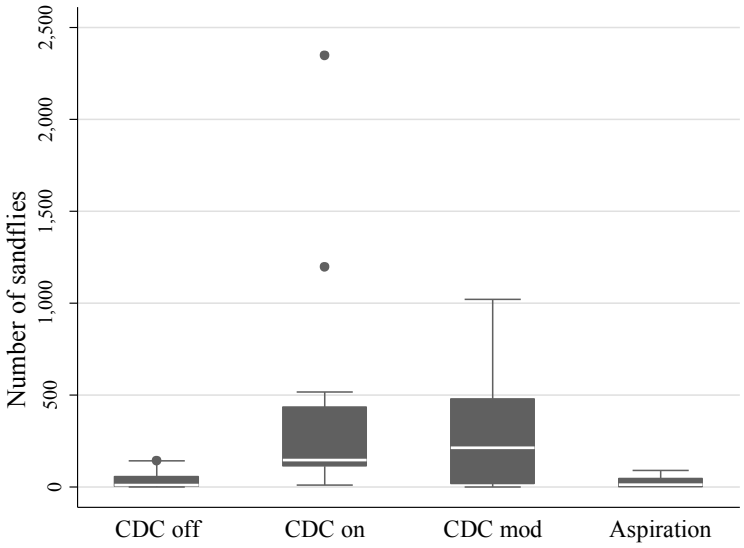


Figure 3.6: Average number of sandflies found upon chickens by four different trap types after an outlier of 7256 sandflies caught in a single night by CDC_{ON} is removed. Median fly densities are represented by white lines. Whiskers and box limits represent the 95th percentiles and interquartile ranges respectively.

Five significant covariates and one borderline significant covariate are associated with number of flies caught on chickens within the mixed effect model (Table 3.8). The forward stepwise inclusion of significant covariates, selected for non-collinearity and biological plausibility, results in the following full model (Table 3.9), whereby trap types and the number of dogs are the only fixed effect predictors which remain in the model.

Table 3.8: Significant predictors of sandfly density upon chickens, when included as single covariates with trap type in the fixed portion of the mixed effects model.

Variable	*Categories*	*Co-efficient (95% CI)*		*SE*	*z*	*P>z*
No. of dogs		0.26	(0.15, 0.37)	0.06	4.66	<0.001
No. of children		0.53	(-0.08, 1.13)	0.31	1.70	0.089
Wall type	Mud					
	Brick	-2.00	(-3.63, -0.37)	0.83	-2.41	0.016
Bednet Y/N	No					
	Yes	-2.00	(-3.63, -0.37)	0.83	-2.41	0.016
Local vegetation type	Trees					
	Manioc	2.00	(0.37, 3.63)	0.83	2.41	0.016

Note: all variables tested individually in a model as fixed effects along with trap type, with house ID and trap night as random effects. All presented variables are significant (P≤0.05) or borderline significant (P≤0.1).

Table 3.9: Full mixed effects model of log fly density upon chickens, taking into account data structuring.

Fixed effects	*Category*	*Co-efficient (95% CI)*		*SE*	*z*	*P>z*
Trap type	CDC_{OFF}					
	CDC_{ON}	2.41	(1.23, 3.59)	0.60	4.01	<0.001
	CDC_{MOD}	2.16	(1.01, 3.32)	0.59	3.67	<0.001
	Aspiration	-0.49	(-1.88, 0.89)	0.71	-0.70	0.485
No. dogs		0.26	(0.15, 0.37)	0.06	4.66	<0.001
Intercept		1.56	(0.6, 2.53)	0.49	3.17	0.002

Random effects	Estimate	(95% CI)	SE
House ID	0.22	(0.01, 7.58)	0.40
Trap night	0.59	(0.19, 1.8)	0.34

3.4.5. Comparison of trap bias upon dogs

To quantify the trapping bias generated by sandfly attraction to light upon dogs, the geometric mean sandfly number per house and peridomestic location was calculated for each trap type and the average density by CDC_{ON} and CDC_{MOD} regressed against average density caught by CDC_{OFF} (Figures 3.7, 3.8).

Simple linear regression of the averaged data demonstrates that CDC_{ON} catches 0.97 log times more sandflies per night than CDC_{OFF} (*β=0.97, T=4.50, P=0.001*). An intercept significantly different from zero (*β=1.87, T=3.52, P=0.006*) also highlights a baseline differen$_c$e in sandfly densities caught by the two trap types, whereby CDC_{ON} recruits an additional 5.42 flies to the trap per night compared to CDC_{OFF}, which lacks the light stimulus. By contrast, CDC_{MOD} recruits 1.17 log times more flies per night than CDC_{OFF} (*β=1.17, T=8.03, P<0.0001*), but attracts only 3.03 additional flies to the trap at the baseline (*β=1.12, T=3.12, P=0.011*). Despite the apparently lower trapping efficiency of CDC_{ON} compared to CDC_{MOD} when considered across all hosts, the level of additional baseline attraction to CDC_{MOD} is lower than to CDC_{ON}, indicating that CDC_{MOD} is less biased.

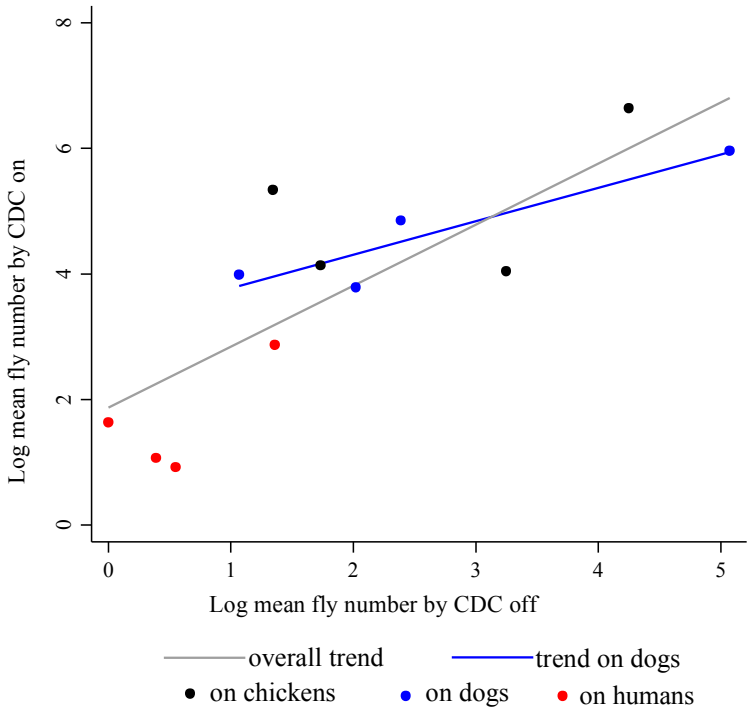


Figure 3.7: Relationship between the geometric mean sandfly numbers caught upon humans, dogs and chickens at each household by CDC_{OFF} compared with CDC_{ON}.

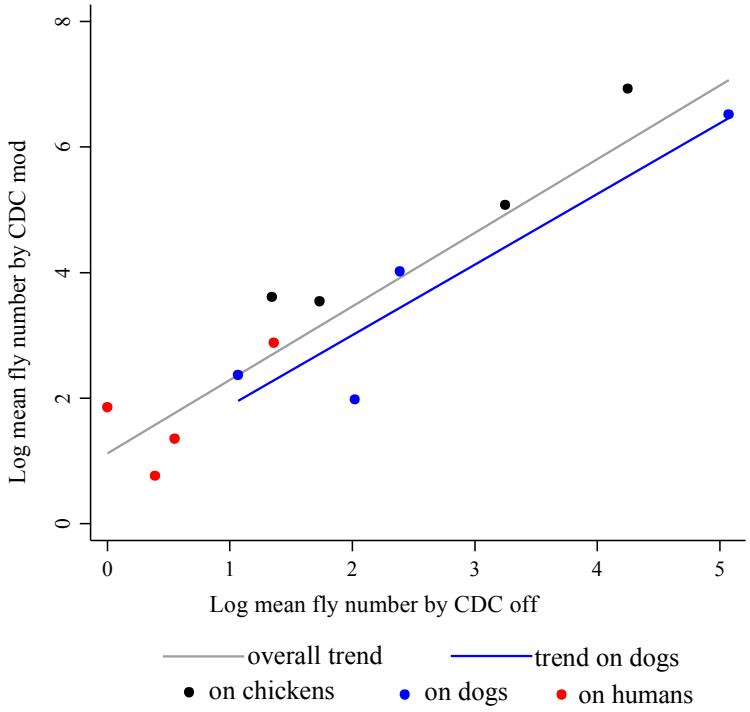


Figure 3.8: Relationship between the geometric mean sandfly numbers caught upon humans, dogs and chickens at each household by CDC_{OFF} compared with CDC_{MOD}.

When the trapping rate upon dogs alone is considered the difference in baseline bias becomes more marked with trapping on dogs by CDC_{MOD}, recruiting a non-significant 2.11 flies (β*=0.75, T=0.83, NS*) but CDC_{ON} recruiting 25.5 more flies to the trap per night at its baseline than CDC_{OFF} (β*=3.24, T=6.88, P=0.021*), indicating significant light bias.

3.4.6. Trap optimisation on humans: human landing catches

The numbers of flies caught by either HLC or indoor CDCLT during the 2.5-hour trapping periods were very low, averaging a geometric mean of just 1.46 sandflies (range 0-43) per CDCLT and 0.92 sandflies (range 0-38) between two HLC workers. Negative binomial regression, taking household ID into account as a random effect

reveals that there is a significant relationship between the densities of flies caught by these two methods (*β=0.24, z=2.27, P=0.023*). This indicates that CDCLT are able to give an indication of the number of flies upon humans and labour intensive HLC may not be necessary to investigate the dynamics of fly density on humans.

3.4.7. Night-time sandfly activity

The numbers of flies caught on all hosts per household were compared for three different periods of capture using a linear regression model with timing as the fixed effect categorical predictor. Compared to the first trapping period from 6.30-9.15pm the number of flies caught during the second period, which ran from 9.15-midnight, was not statistically different (*β=0.04, z=0.19, P=NS*). The third trapping period, midnight-6am, however, caught significantly more sandflies (*β=0.87, z=4.17, P<0.0001*). Given the third trapping period ran for twice the time of the others this is perhaps not unsurprising. However, when the catches from the two initial periods are summed together and the regression rerun, there is no significant difference between the capture rate by time periods (*β=0.14, z=0.83, P=NS*). This demonstrates there is no significant difference in capture rate during the first and second half of the night, nor between the first and second quarter of the night. Therefore, there does not appear to be a period of peak fly-biting activity.

3.5. Discussion

Four different methods of trapping sandflies were compared. The aim was to estimate trapping efficiency and bias upon dogs, humans and chickens.

Evaluation of the four trapping methods confirmed that CDCLT with the light on caught the most sandflies on all three hosts. Standard CDCLT are effective because *Lu. longipalpis* sandflies are sensitive to the visible spectrum (Mellor *et al.*, 1996), exhibit a positive photo-taxic response and are actively recruited to the traps (Alexander, 2000; Alexander and Young, 1992).

By comparison, direct aspiration and CDCLT without light caught the fewest sandflies on all hosts once confounding factors had been taken into account. In the case of aspiration this is due in part to the restricted trapping period of just 40 minutes in total compared to the other methods. However, direct count observations are generally suboptimal when density is low or very high, even by trained operators (Mathenge *et al.*, 2005; O'Meara *et al.*, 2006). This means that the reduction in fly count due to time limitations may be exacerbated for low density locations or nights, such as inside houses, where there are very few flies dispersed over a large, complex area (Quinnell and Dye, 1994b). Alternatively, when density is very high, maximal aspiration rate may be insufficient to collect all the flies within the time. The challenges of aspiration also differ upon each host, as the size of the sample area, habitat complexity and degree of sandfly aggregation differs between locations and nights (Alexander, 2000). When sandflies aggregate, usually upon outdoor dwelling hosts (Quinnell and Dye, 1994a), it contributes towards ease of capture in these locations, which is an obvious source of bias between hosts when comparing captures.

CDCLT with no light catch the fewest flies as they rely on sandflies being active in very close proximity to the trap (Muirhead-Thompson, 1991). CDCLT with light improve on this design, particularly inside houses and chicken sheds where sandflies can be attracted to the trap following attraction into the structure in response to host stimuli (Quinnell and Dye, 1994b).

However, as shown by the linear relationships between the numbers of flies caught by the different CDCLT methodologies, there appears to be significant trapping bias on dogs associated with light. Higher basal capture rates by CDC_{ON} traps compared to CDC_{OFF} are indicative of recruitment to a light source in excess of that explained by increases in efficiency, which indicates bias. This bias is most evident on dogs, likely because dog traps were placed in relatively exposed outdoor locations, typical of dog sleeping habits on Marajó (Quinnell and Dye, 1994a). Therefore the light from the CDCLT is not limited to the confines of a house or shed. Instead, the area over which flies are recruited to the CDCLT is influenced by the responsiveness of flies to light over distance (Alexander and Young, 1992), creating a major source of bias when calculating proportional host preference between enclosed and exposed hosts.

By comparison, CDCLT modified by the addition of a hood were associated with the second highest number of flies once confounders had been taken into account. Additionally, CDC_{MOD} appeared to be relatively unbiased on dogs compared to CDC_{ON}. Here, the baseline number of flies per trap is lower by CDC_{MOD} than CDC_{ON}, indicating a reduction in the added attraction to the CDC_{MOD} due to the reduction of the light stimulus. CDC_{MOD}, however, remains efficient in comparison with CDC_{OFF}.

Hoods limit the direction of the light source to directly above the host, which permits light based attraction of flies visiting the host only. Hooded CDCLT have been successfully used to increase capture rate in the absence of light stimuli by funnelling flies into the trap (Campbell-Lendrum *et al.*, 1999b). The modification presented here improves further upon this design, utilising both the funnelling effect and light stimulus to maximise capture while preventing light from biasing preference.

Furthermore, the average fly density within houses appeared to be higher on nights when modified CDCLT were used on dogs compared to standard CDCLT trapping nights, though the difference was non-significant. This is another possible indication of bias due to light in CDC_{ON} traps whereby flies are attracted toward the exposed light source on dogs, at the expense of recruitment to humans. Conversely, the average density per chicken shed was highest on nights with standard CDCLT on dogs. Again, though this difference is not statistically significant it may be an initial indication of another push-pull, competitive process. Hypothetically, general attraction to the peridomestic environment due to an additional light source on dogs may increase local fly density, and subsequently recruitment to highly attractive hosts such as chickens. General increases in sandfly density in the peridomestic environment have been suggested following the extensive electrification of houses (Barghini and de Medeiros, 2010), but further investigation is required to confirm if these effects are significant.

There was also significant nightly variation in capture rate which is likely to be explained by environmental factors. On dogs the difference between nights appears to be almost entirely due to the significant effects of wind strength. In fact, wind was

a significant confounder of trap efficacy upon dogs; so that modified traps only became the second most efficient capture method after wind had been taken into account. Weather is an accepted predictor of sandfly densities (Morrison *et al.*, 1995a; Ximenes *et al.*, 2006). Wind may be particularly important as sandflies are weak fliers (Dias-Lima *et al.*, 2002), meaning fewer flies are on the wing and available for capture on windier nights, especially to traps raised above the ground (Faiman *et al.*, 2009). Moreover, there may be a reduction in suction and trap efficiencies at high ambient air speed. The impact of wind is likely to be marked upon dogs due to their exposed location in contrast to hosts inside semi-enclosed structures; hence wind was a significant confounder of fly density only upon dogs, and explained the majority of variability between nights.

Of the other significant predictors of fly density upon the three hosts, none confounded the effect of trap type. On dogs the full model shows that in addition to trap type and wind, sandfly density was also positively associated with *M. esculenta* cultivation. Quinnell and Dye (1994a) noted a similar association between high fly densities and cultivation, potentially indicating the importance of certain vegetation and soil types in sandfly population maintenance. However, vegetation type in the data presented here was highly collinear with numerous other significant predictors and perfectly collinear with wall type and bednet usage, which makes interpretation difficult. Local vegetation type may not represent a causal relationship with sandfly density but instead be symptomatic of constant differences between households. Only four households were used in this investigation; therefore, household predictors are likely to be highly collinear with each other and house ID. This was confirmed by the reduction in the amount of variation attributable to random effects of house ID

following the inclusion of household level predictors (Rice *et al.*, 1998). It is therefore difficult to draw firm conclusions regarding the importance of household level variables and how they can be meaningfully interpreted.

For example the covariates of dog and duck density were positively related with sandfly catch on chickens and humans respectively, and both on dogs. Specific host densities may be important predictors of sandfly density, but as they are collinear with household this finding must be viewed with caution. The association with household does, however, offer an explanation for counter intuitive results, such as why human density at the house trap was negatively associated with fly catch and increasing distance between hosts was associated with higher vector densities. These variables are simply indicative of different households. Consistent differences between households may be partially explained by the above predictors or constant factors such as proximity to resting and breeding sites and possible sandfly site fidelity. Yet given that only four houses were under observation discussions regarding these covariate risk factors must remain speculative.

Capture rates by all methods within the house yielded lower densities than those upon dogs and chickens. Such low densities indoors are not unusual (Courtenay *et al.*, 2007; Quinnell and Dye, 1994a), and help to confirm that *Lu. longipalpis* are not highly anthropophilic or endophilic (Alexander, 2000). Human behaviour at night time in comparison to animals may also be a contributing factor, specifically, people are not always in close proximity to the trap of interest and they reside within well-constructed houses which may restrict sandfly access (Quinnell and Dye, 1994b). Importantly, however, the number of flies caught by CDCLT and HLC over 2.5 hour

periods appear to be related, indicating non-labour-intensive CDCLT may be used to estimate the number of flies visiting humans. The relationship is not, however, directly proportional, which shows that HLC may catch slightly more flies than CDCLT. Nevertheless, the highly intensive nature of HLC, acceptability to householders and the limitation they may impose upon trapping periods are prohibitive to their extensive use. Additionally, the necessary presence of operators for the HLC means that host density is artificially increased by this method.

Comparison of capture rates over periods of the night confirm that flies can be easily caught at all times throughout the night. This is in stark contrast to the activity patterns previously reported for Marajó sandfly populations, which show a peak in activity in the early evening, and a significant drop in flies caught after midnight (Courtenay *et al.*, 2007; Kelly and Dye, 1997). This is indicative of changing trends in sandfly activity, possibly related to the increased electrification of households which encourages prolonged activity close to houses (Barghini and de Medeiros, 2010). It follows therefore that assessment of sandfly preference should utilise night-long trapping in order to capture the total variation in biting rate and preference and maximise sample size.

Overall the findings of the current investigations confirm regular differences in the biting rate between three hosts of interest and represent the first attempt to compare this combination of CDCLT and aspiration techniques in the field, including the development of a new trapping protocol to assess efficiency and the bias effect of light on sandfly capture. Night-long use of modified CDCLT on dogs and standard CDCLT on chickens and humans appear to offer the best option for the investigation

of sandfly biting preferences across multiple hosts and peridomestic locations. However, there are limitations within the data that must be made clear.

Firstly, when trapping inside chicken sheds and houses it is possible that resting sandflies may be caught in the trap in addition to host seeking insects of interest. Given that few sandflies are found in these locations during the day (Brazil *et al.*, 1991; Morrison *et al.*, 1993a) and that *Lu. longipalpis* is not a highly endophilic species (Campbell-Lendrum *et al.*, 1999b), it remains reasonable to assume that flies which have been attracted to the site by the animal bait and sex pheromone each night anew.

Secondly, relative sandfly numbers captured near hosts are only a proxy for biting preference. Alternative host-independent methods are a crucial tool for validation of the presented capture methodology. Forage ratios from bloodmeal analyses could be used to validate host preference (Morrison *et al.*, 1993b), despite being very expensive and time consuming for the regular assessment of host preference in the field. However, like the methods presented here, capture of flies for bloodmeal analysis is also subject to significant issues of capture bias that need to be overcome. One possibility may be to collect flies from a range of non-host related resting sites.

Calculating the bias attributable to trap type is a challenge. Here, the average density per host per household for each trap type was compared by regression. Averages were used because the individual densities caught by different traps on different nights are not directly comparable. The loss of individual variation generally leads to stronger correlations than would be seen in individual based data (De Veaux *et al.*,

2010; Moore *et al.*, 2010), plus the averaged dataset is relatively small, meaning caution needs to be exercised in interpretation. Nevertheless, the difference in nightly capture rate on dogs due to light appears to be large. When considered in the framework of current understanding of sandfly trap behaviour, the modified trap appears to be the least biased trapping method whilst maximising efficiency. The use of non-rotational experimental designs may permit the use of nightly count data in comparison of trap type in any further investigations. In retrospect, given more logistical resources, using the modified CDCLT away from any hosts would have added additional comparison data and provided a control to host effects.

Ideally, total fly density per host would also be calculated in order to estimate better the efficiency and bias of each trapping method. However, population knock-down or capture (such as the drop net captures of Jones and Quinnell (2002)) were impractical here, and estimate the number of flies at a particular time, rather than reflecting the number of sandflies visiting a host over the course of a night.

Finally, the number of trapping nights by aspiration was low in comparison to by the CDCLT methods, which means the experimental design is unbalanced. This may have contributed to the apparent inefficiency of aspiration in comparison to other techniques on dogs.

In future, the inclusion of more houses could improve the power of the study, in particular to detect competitive (push-pull) interactions between hosts, and additional trap methodologies could be incorporated, such as baited bednet traps upon people in

additional to HLC and CDCLT, and differential positioning of CDCLT. However, limits on time and equipment prevented a larger scale preliminary investigation.

3.6. Conclusions

To assess behaviour and relative density of sandflies on different hosts it is necessary to reduce sampling bias without sacrificing sample size. Here, is it demonstrated that standard CDCLT are a highly effective way to catch high numbers of sandflies, particularly on chickens and humans. Yet, CDCLT are subject to light-related bias in open environments. It is therefore proposed that the CDCLT modifications presented here reduce bias, and provide a more accurate estimate of the numbers of sandflies visiting dogs, the important reservoir host, per night. The development of these reduced trapping bias methodologies provides the opportunity to investigate vector host preference under a range of conditions, specifically over a range of vector densities, which as hypothesised in Chapter 2, may be an important determinant of host preference.

Chapter 4: **Vector density: measurement, manipulation and effect on host choice**

4.1. Introduction

Multiple hosts are implicated in visceral leishmaniasis transmission in the New World; dogs are the main reservoir of zoonotic VL whereas infected people are considered "dead end" hosts. Yet, within the peridomestic environment there are a variety of other non-competent hosts present on which vectors of leishmaniasis, such as *Lu. longipalpis,* readily feed (Morrison *et al.*, 1993b; Quinnell *et al.*, 1992). The presence of multiple blood sources within the peridomestic environment means that many animals can serve to maintain the vector population, which presents an obvious challenge to sandflies in that they must make decisions regarding host choice. Variation in host preference and the biting rate between hosts can have important consequences for patterns of disease transmission, as indicated by initial modelling and as hypothesized in Chapter 2, and therefore this warrants further investigation in the field.

In general, sandflies are considered to be catholic and opportunistic in their biting preference (Quinnell *et al.*, 1992). Blood meal analyses have revealed *Lu. longipalpis* have a wide host range including dogs, humans, wild animals, such as opossums, in addition to domesticated animals and livestock (Morrison *et al.*, 1993b).

Studies in Colombia have shown that large animals such as bovines and pigs are the predominant blood source for *Lu. longipalpis* (Morrison *et al.*, 1993b), potentially highlighting an innate preferences of sandflies for these specific host types. However,

the over representation of these hosts in the aforementioned study may by symptomatic of bias in their trapping methods, whereby aspiration took place only on specific hosts. More generally, trap positioning with respect to the nearest host undoubtedly influences the trapped sandfly blood sources.

Host biomass and access are reported as key determinants of host preference (Quinnell *et al.*, 1992). Under this view, long-range attraction of hosts to sandflies is on the basis of biomass, likely due to greater semiochemical production corresponding to body size. Male sandflies also emit a sex pheromone when lekking in association with a host which acts in synergy with host odour (Bray and Hamilton, 2007b) also to attract females to the host. Therefore the distribution of males between hosts is also pivotal in determining realised biting rates (Kelly and Dye, 1997) and may result in density dependence, whereby host choice is driven by the density of sandflies, and the density of sandflies is influenced by density of hosts. Additionally, it is not clear to what extent host preference changes either over space or time.

From mathematical modelling presented previously (Chapter 2) it is proposed that human and canine infection prevalence patterns may be explained by variation in host choice with vector density. Key model hypotheses were that the relative preference for alternative hosts, such as chickens, would increase with vector density and correspondingly reduce upon dogs and humans, despite possible absolute increases on all host types.

Abundance of sandflies could potentially be manipulated in a number of ways, such as via the application of insecticide. However, insecticide may induce repellent effects and the disruption of pheromone based aggregation in sandflies (Kelly *et al.*, 1997), thus effecting sandfly distribution. Less disruptive alternatives include the removal of vectors by excessive trapping, such as has been used for the successful control of other disease vectors, like *Glossina palpalis,* the vector of human African trypanosomiasis on the Island of Principe in 1910-1914 (Molyneux, 2006), and has been occasionally observed during the intensive capture of sandflies for other purposes (Arias and Freitas, 1982; Courtenay, pers. comm.)

Abundance of *Lu. longipalpis* is also known to fluctuate naturally across the wet and dry seasons in response to environmental changes, in particular rainfall, relative humidity and temperature (Morrison *et al.*, 1995a; Morrison *et al.*, 1995b) and wind (Ximenes *et al.*, 2006). The periodicity of seasonal variation, however, differs across the geographical range of *Lu. longipalpis*. For example, peak *Lu. longipalpis* densities in the Brazilian states of Mato Grosso do Sul occur in February and April (de Oliveira, 2008), but occur mainly in February in Minas Gerais (Resende *et al.*, 2006), May in Rio Grande do Norte (Ximenes *et al.*, 2006; Ximenes *et al.*, 1999) and June in other Brazilian regions (Deane and Deane, 1962; Galati *et al.*, 2003). These peaks in density were reached between 3-8 weeks after peaks in rainfall (de Oliveira, 2008; Morrison *et al.*, 1995a). By contrast, in other regions of the *Lu. longipalpis* geographical range, such as in regions of Costa Rica (Zeledon *et al.*, 1984), and Marajó (Kelly *et al.*, 1996), vector densities are reported to peak at or prior to the commencement of seasonal rains, possible as humidity increases, more closely reflecting the dynamics of Old World sandfly species such as *P. argentipes* (Dinesh

et al., 2001). Differences in seasonal trends between *Lu. longipalpis* populations may be partially due to differences in local climatic conditions and the nature of seasonal change in differing ecotopes, but is also potentially a result of differences in the response of different *Lu. longipalpis* subspecies to climatic variables (Morrison *et al.*, 1995a).

Seasonal patterns can be highly variable between years and locations (de Oliveira, 2008), even resulting in non-detectable seasonal change in some years (de Oliveira *et al.*, 2003). In regions of low sandfly density, detection of seasonal trends can require larger capture sample sizes, as illustrated by the studies of Resende *et al.* (2006) and Queiroz *et al.* (2009) where sampling effort was low and only low densities recorded, making it difficult to separate the immediate effects of rain and humidity from longer term seasonal variations.

Overall, host preference remains poorly understood with simple linear preference-vector density relationships being conventionally assumed in current models, i.e. doubling the density of sandflies (parameter V in equation 2.3, Chapter 2) will double the effective biting rate ($a\theta_D V/D$) on dogs and have no effect on vector preference (θ_D would remain constant).

4.2. Aims

Following the hypothesis of nonlinearity in transmission and biting rates to chickens, dogs and humans put forward using mathematical models (Chapter 2), the aim of this

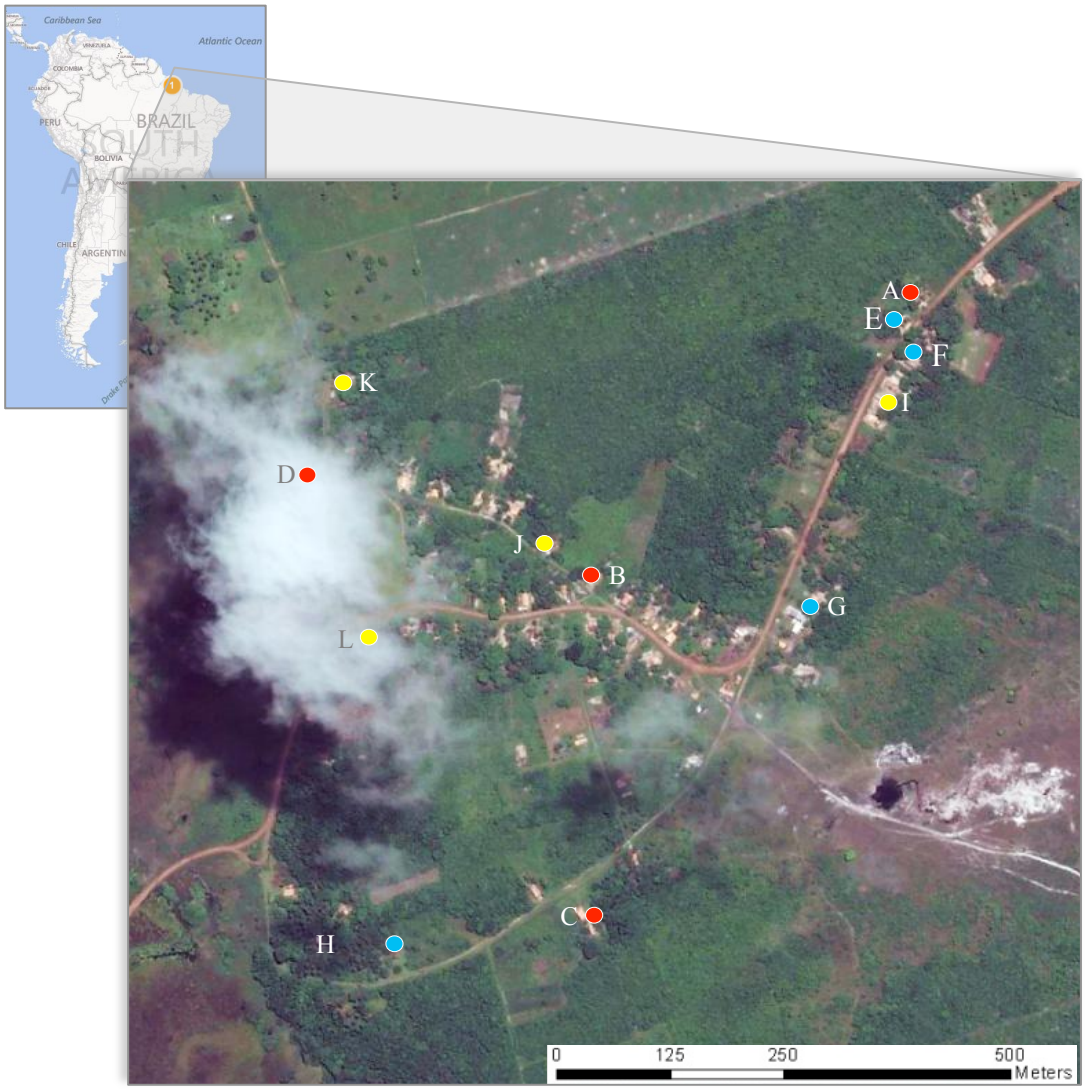
chapter is to test the hypothesis that host choice in *Lu. longipalpis* in the field is influenced by vector density.

1. Testing this hypothesis in the field was achieved by addressing the following specific objectives:
2. Experimentally manipulate local vector density via "trapping out" to observe the relative density of sandflies upon dogs, humans and chickens over a range of vector densities.
3. Observe the relative density of sandflies upon dogs, humans and chickens over a natural period of vector density variation caused by seasonality.

4.3. Methodology

4.3.1. Study Area

All experiments were carried out in the village of Boa Vista (BV), a large village in the Salvaterra district of Marajó Island, Pará State, in northern Brazil (Figure 4.1). BV was selected due to its relatively high vector density (Roberto Bahia, pers. comm.; Quinnell and Dye, 1994a), and supported high density of clinically sick dogs observed in the village by the project team (Courtenay, unpublished), and local Secretaria de Saúde.



Houses A-D enrolled for both "trapping out" vector manipulation and observation over seasonal variation (experiments 1 and 2).

Houses E-F enrolled for observation over seasonal variation (experiment 2).

Houses I-L enrolled for host density manipulation (Chapter 5).

E and F Paired close proximity houses for comparison (see Section 4.3)

Figure 4.1: Aerial photograph of the study village, BV (48°34'36.27"W, 0°48'4.64"S), and the location of houses enrolled into each study. Image adapted from Bing Maps aerial imagery (Microsoft Corporation 2010, NAVTEQ 2010, Intermap 2010).

An initial three nights' trapping were carried out in all 12 consenting households within the village which met the selection criteria of having a resident human, dog and chickens which roost within a chicken shed. The four consenting households that had the highest geometric mean fly density were selected for inclusion in experiment 1, the "trapping out" study (see Figure 4.1 for location of all trapping houses). Of the remaining consenting houses, the four with the next greatest geometric mean were selected for experiment 2, the seasonal variation study. Upon the completion of experiment 1, the experiment 1 houses were enrolled for delayed inclusion into experiment 2. Of all the enrolled houses, capture data from the two households in greatest proximity on the same trapping regime (houses E and F) were used to explore spatial relationships.

4.3.2. Collection Protocol

As previously described in Chapter 3, traps were set in three domestic locations in close association with each host of interest; one trap in the bedroom of the house, one in the chicken shed and one in association with a caged dog to sample the number of flies visiting these three host species. Trap position was optimised for each individual trap location, with the trap entrance being positioned as close to the host as possible with standard CDCLT light trap capture occurring in the house and chicken shed and modified CDCLT light traps being used on caged dogs to maximise sandfly capture rate upon all three hosts and reduce trapping bias (Chapter 3). Traps and hosts were located equidistant from one another; ensuring dogs were caged at a distance from the house and chicken shed equal to the distance between the two. This removes

distance as a potential confounder in fly preference and reduces the proximity of dogs to potential confounders such as outside lights and domestic facilities.

Traps were set at 6.30pm and collected at 6.30am. CDCLT bags were changed at 9.15pm and midnight as part of another experiment into sandfly activity over the course of the night, but all closed CDCLT bags were left hanging next to the CDCLT trap so as not to disrupt pheromone communications and associated sandfly recruitment (i.e. bags contained live flies).

Upon final collection, flies were transferred to ethanol. The dead sandflies were identified and separated by sex before being counted under a dissection microscope as described in Chapter 3.

4.3.3. Study design

Experiment 1: *Experimental "trapping out".*

Ten consecutive nights of CDCLT capture upon all three domestic hosts were carried out in order to deplete experimentally local vector density through the practice of "trapping out". Vector density and distribution between the three hosts of interest was observed over this period of excessive trapping in order to estimate the effect of local population reduction upon preference. Following 10 days of expected depletion, captures were then carried out once every three nights for a further 30 days in order to observe sandfly distribution and density upon hosts during sandfly expected recovery, giving an equal number of trapping nights both during the decline and

recovery phases of the experimental manipulation. Ten days was expected to be sufficient for vector depletion, as continuous trapping in the same locations has been previously observed to impact rapidly upon capture success (Orin Courtenay, pers. comm.).

Experiment 2: *Seasonal variation in vector density and preference.*

Sampling of vector density on all three hosts was carried out in a total of eight households during November 2011 and from January to the end of July 2012 at least once a week in order to observe sandfly density and distribution over a period of natural seasonal variation in vector density. Beginning in January 2012, houses A-D were additionally sampled for 10 consecutive nights, and biweekly for a further month (as outlined in experiment 1 above). Due to limited equipment and transport availability, it was not possible to trap in December or the first three weeks of April (29.11.11-04.01.12 and 28.03.12-21.04.12).

Intermittent rains began in January, progressing to nightly rainfall during the peak in April, returning to the dry season by the end of May. Subjective measures of rainfall (scale of 0-3 indicating none, light, moderate and heavy rainfall) and wind (scale of 0-5 for none, very light, light, moderate, strong and very strong wind) were recorded on each trapping night, later expanded to measure mm of rainfall per trapping nights from the end of February 2012.

4.3.4. Analysis

All count data were log transformed [*ln(n+1)*] for parametric analyses, and analysed in STATA v11.0 (StataCorp LP). Where any trap had failed or dog escaped during the capture period or night, the specific house-night data were excluded from the analysis.

Analyses of vector density were performed using simple linear regression and mixed effect models, incorporating household ID as a random effect. Similarly, basic analyses of proportional data were carried out using GLM specifying a binomial distribution and the logit link function, and also GLMM to permit the inclusion of house ID as a random effect in order to take into account structuring within the data due to repeated measures on household.

Spatial relationships were also examined in brief by comparing sandfly densities in two closely situated households using simple linear regression.

4.4. Results

4.4.1. Trapping out

Following the application of exclusion criteria a total of 61 house-nights data were collected over 8 houses in order to investigate vector preference over a range of fly densities. A total of 28 house-nights of data were collected corresponding to the intense "trapping out" phase. When all house-night data are taken as a whole there appears to be a small decrease in the number of sandflies caught over the period of

intense trapping (Figure 4.2), which indicates possible but limited success of the trapping intervention to manipulate host density.

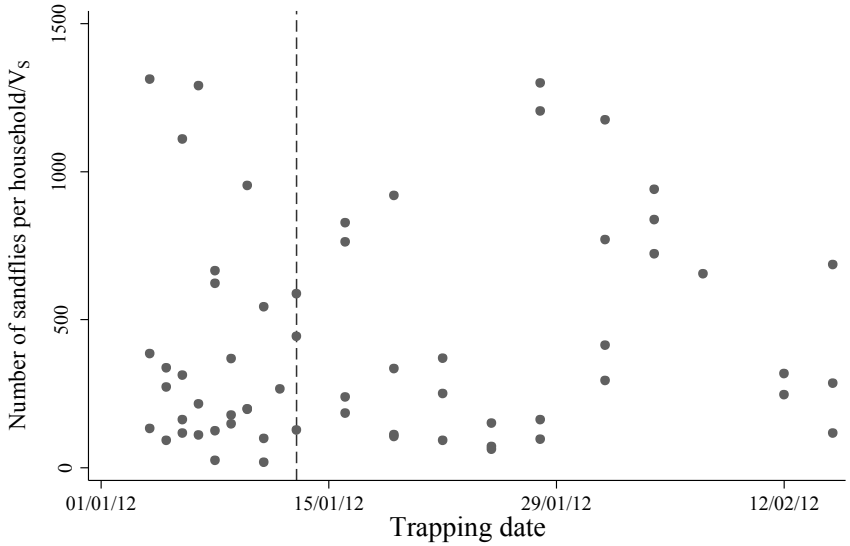


Figure 4.2: Nightly variation in sandfly density during "trapping out" and recovery phases of experiment 1; whereby the vertical dashed line demarcates the end of the "trapping out" intervention. Each point represents one house-night.

Overall, however, the reduction appears to be small while variation between nights is large. Additionally, despite clear differences in sandfly density between households all houses experience large variation in vector density, and household level trends also fail to demonstrate a reduction and recovery of vector density associated with the intervention (Figure 4.3). Statistical analysis of these data including house ID as a random effect confirms the non-significant decline in vector number over the course of "trapping out" (β*=-0.010, z=0.73, P=0.463*).

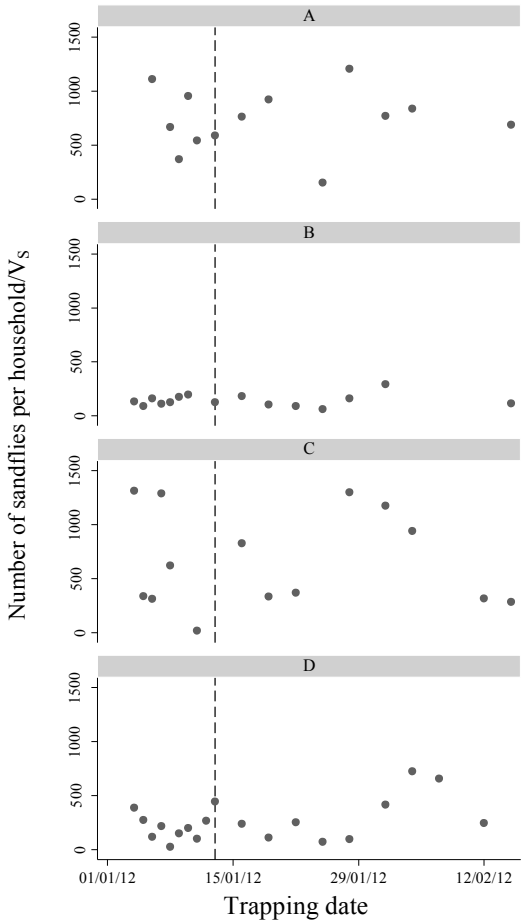


Figure 4.3: Nightly variation in sandfly density for each house during the "trapping out" and recovery phases of experiment 1, whereby the dashed line demarcates the end of the intense trapping intervention period.

Given the failure of "trapping out" to influence vector density over the course of the observation period, count data from this experiment were incorporated into the seasonal variation dataset in order to maximize the number of sample points throughout the trapping period.

4.4.2. Seasonality in vector density and preference

Observation across an 8-month period of seasonal change culminated in a total of 203 house-nights of valid collection data from 8 houses. This resulted in the capture

and separation of over 22,201 females and 46,218 male sandflies. The distribution of fly counts was overdispersed and skewed to the right, with total household capture (the sum of fly counts caught across all three hosts) ranging between 11-2356 flies per household per night, with a geometric mean of 216.47. Gaps in these data (December and April) correspond to periods when it was not possible to trap.

Household vector density (the sum of fly counts across hosts) appears to vary significantly over time, with local household vector density reaching its peak in January, declining after the beginning of the rains in February to reach its lowest in June (Figure 4.4a). The raw count data are highly overdispersed, but log transformation normalises the variation. Linear trends confirm a general reduction in household sandfly densities over the course of the observation period *(β=-0.005, z=-6.05, P<0.0001)*, particularly from the January peak in fly density as the season's transition from the dry to wet (β=-0.008, z=-7.70, P<0.0001) (Figure 4.4b).

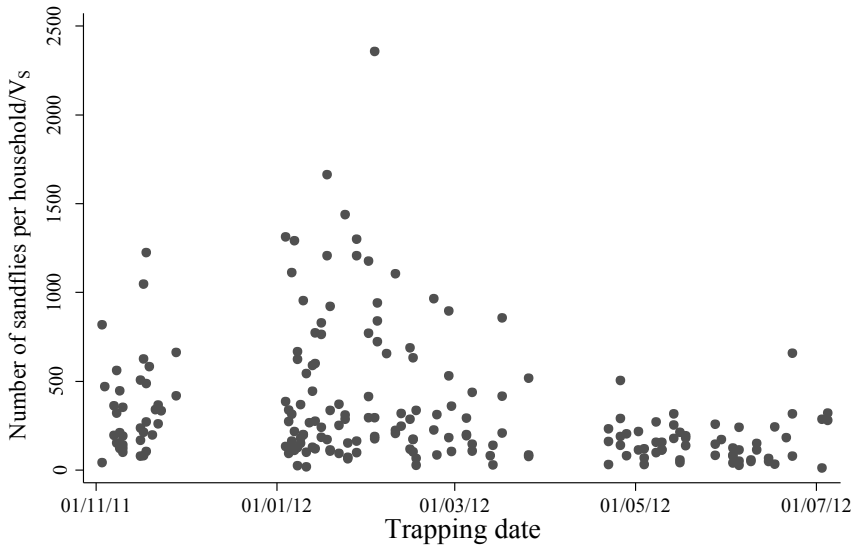


Figure 4.4a: Change in household vector density over a period of seasonal variation. Each point is a house-night.

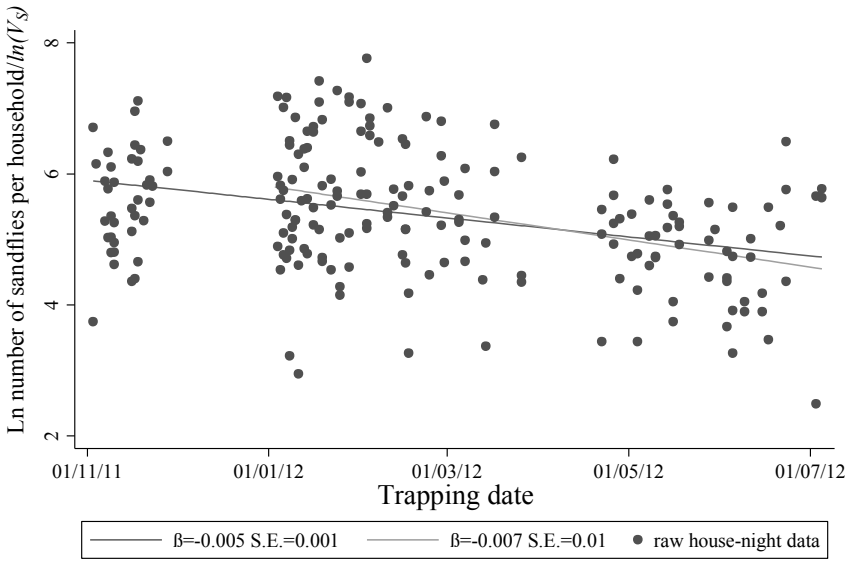


Figure 4.4b: Change in *ln* household vector density over a period of seasonal variation. Each point is a house-night. Each regression line represents the trend in sandfly density over differing periods.

Reduction in vector density over time and the presence of a peak in sandfly density in January 2012 are also detectable at the household level (Figure 4.5). Variations in household vector densities are highly significant both over the full experimental period (November 2011-July 2012) (β*=-0.005, z=-6.05, P<0.0001*) and between the January 2012 peak in abundance and July 2012 (β*=-0.008, z=-7.70, P<0.0001*), after variation attributable to household is taken into account by the inclusion of house ID as a random effect.

Household level variations in vector density over time are also accompanied by variation in the density and preference of sandflies for each host of interest (Figure 4.6). These data indicate a high level of variability in preference between households over time, plotted as fortnightly periods.

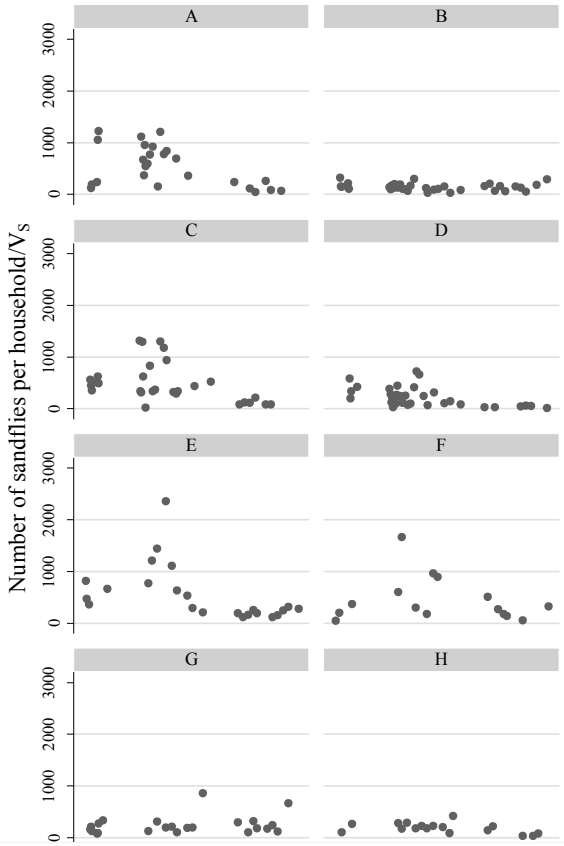


Figure 4.5: The number of sandflies caught across all domestic locations in each household over the period of seasonal change

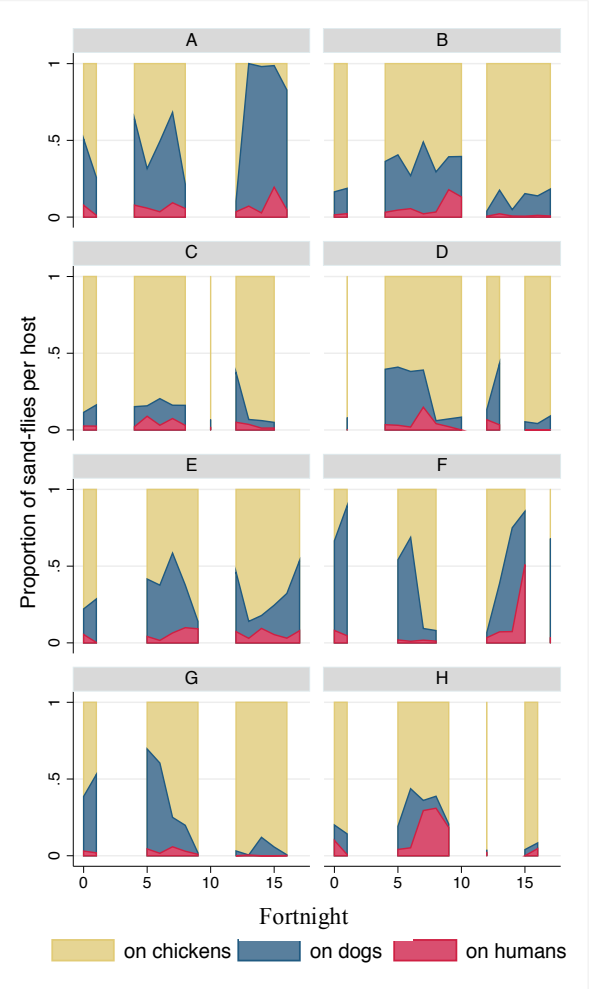


Figure 4.6: The total fortnightly proportion of sandflies caught on humans, dogs and chickens for each household over the season. Gaps represent fortnights with no data.

The actual number of flies caught on each host, chickens (β=*-0.83, z=-3.61, P<0.0001*) dogs (β=*-0.47, z=-3.14, P=0.002*) and humans (β=*-0.001, z=2.54, P=0.011*), significantly reduces over time, once house ID has been taken into account. Associated with reduction in vector densities over the season, there is also a significant increase in the proportional preference for humans over the season (β=*0.001, z=3.85, P<0.0001*), and a decrease in the relative preference for dogs (β=*-0.001, z=-8.33, P<0.0001*), taking into account household effects. However, as these changes in proportional preference do not appear to be very large, they are not easily discernable when the data is plotted (Figure 4.6).

4.4.3. Vector density and preference relationships

In addition to seasonal trends, nightly host preference among female flies appears to be significantly related to nightly vector density (Figures 4.7 and 4.8). Specifically, sandfly preference appears to change nonlinearly over the range of vector densities as predicted by simulated host preferences in Chapter 3 (Figures 2.4 and 2.5), with the proportion of female flies caught on chickens apparently increasing nonlinearly with vector density, and simultaneously seeming to reduce nonlinearly upon both dogs and humans. However, this relationship is not clearly defined within the seasonal data, as there also appears to be substantial nightly variation, producing a "noisy" relationship, especially in the case of dogs and chickens.

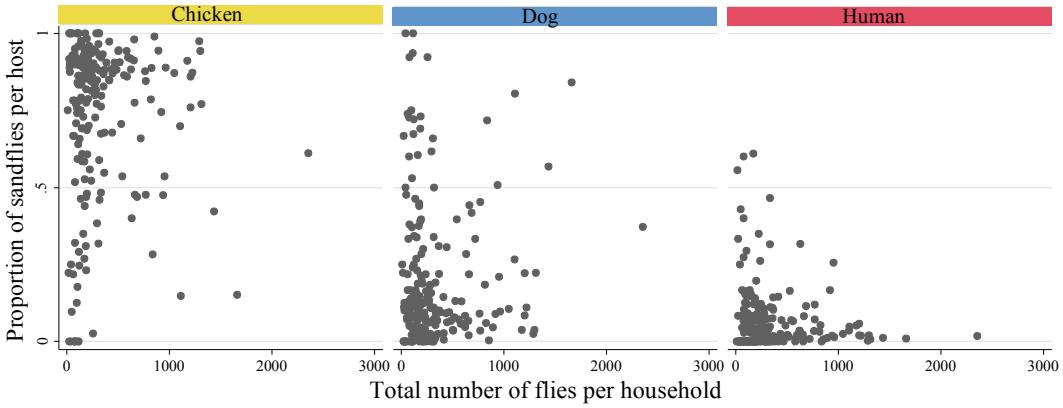


Figure 4.7: The distribution of sandflies between three hosts of interest with vector density. Each point is a house-night observation.

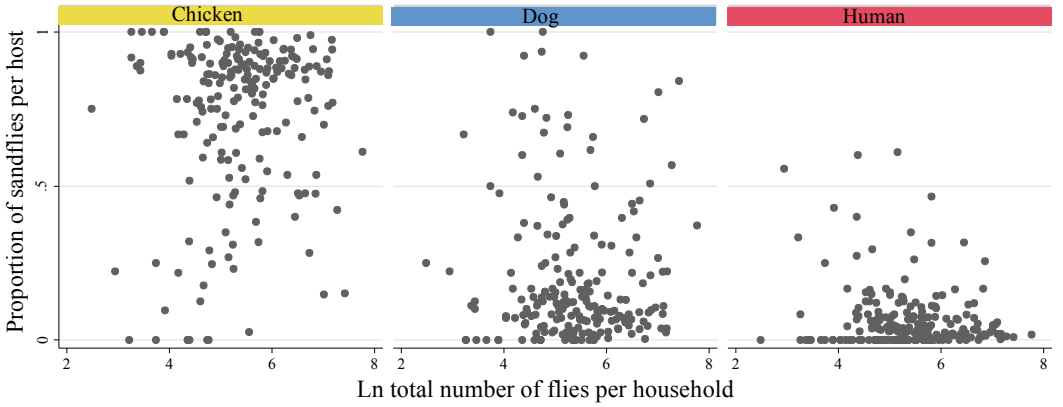


Figure 4.8: The distribution of sandflies between three hosts of interest with *ln* local vector density. Each point is a house-night observation.

If sandfly densities are averaged over time to obtain a monthly geometric mean fly density per household, and the total proportion of flies caught on each host per month, apparent nonlinear trends in preference with vector density remain evident (Figure 4.9). Variation between houses and months continues to be substantial but the relationship between vector density and preference appears to remain stable over differing time scales.

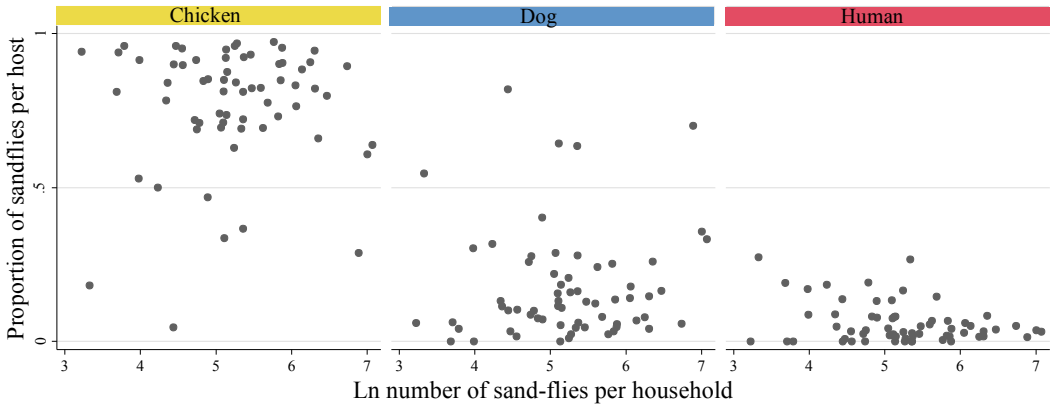


Figure 4.9: Relationship between monthly distributions of sandflies between three hosts of interest with geometric mean local vector density. Each point is a house-month.

By contrast, the nonlinear relationship is less evident when nightly data are averaged across households to give the nightly geometric mean fly density and preference (Figure 4.10). The density of sandflies varies between households on any given night, and by association, so does relative preference, hence averaging over households may not converge to a general nightly pattern.

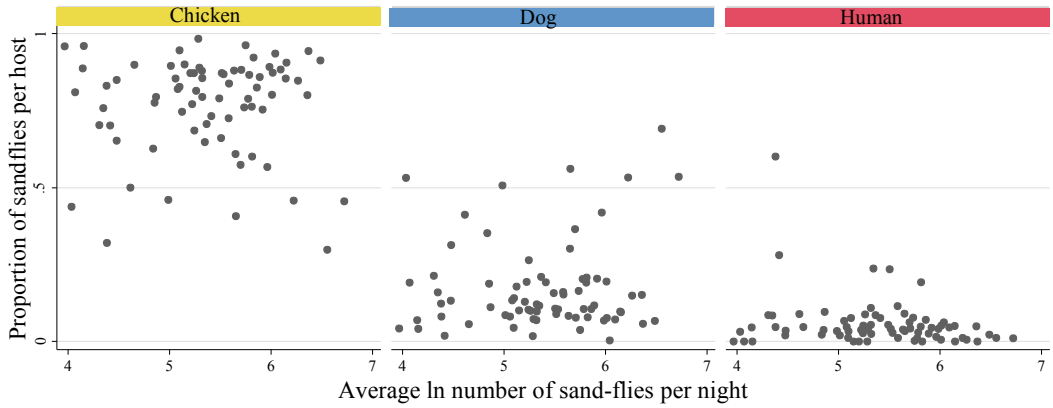


Figure 4.10: Relationship between the distributions of sandflies between three hosts of interest with average logged nightly density across all houses is taken. Each point is an average night observation.

This is further confirmed when the vector densities and preferences occurring in the two most neighbouring households enrolled in the study, houses E and F (Figure 4.1 for location), are considered as an isolated pair. No measure of vector density, including *ln* household catch ($\beta=0.40, T=1.21, P=0.251$), demonstrates a significant relationship between these two households, despite their proximity and being sampled on the same nights. This indicates that vector density-dependent preference dynamics occur on a highly localised scale.

4.5. Discussion

In a series of field experiments and observations, vector densities were (i) experimentally manipulated via sustained CDCLT capture in an attempt to reduce local vector density (also known as "trapping out"), and (ii) observed over a period of seasonal variation, in order to investigate density dependent biting preference of *Lu. longipalpis* sandflies on three predominant domestic hosts.

"Trapping out" may have resulted in a slight reduction in vector density during the period of intense trapping, but on the whole, proved to be an ineffective intervention for manipulating vector densities. This may be due, in part, to overwhelming nightly variation that may swamp any short-term trends in reduction. "Trapping out" has been previously reported as a secondary outcome of intensive trapping of sandflies around Manaus, Brazil, whereby sandflies were caught primarily for the purposes of *Lutzomyia* population survey (Arias and Freitas, 1982). Here, a reduction in the average catch was observed over the first five weeks (Arias and Freitas, 1982). It is possible, therefore, that the period of "trapping out" selected for the collection of the

above data was insufficient to significantly reduce local vector density. This manipulation was reliant upon the removal of adults; however it appears that the emergence rate from pupal stages or immigration of adults from elsewhere was sufficient to replenish the population, despite the intensive trapping effort. Trapping was carried only out for a period of 10 days, much shorter than the average sandfly oviposition to emergence time of 4-6 weeks (Morrison et al., 1995b; Volf and Volfova, 2011). Therefore, any downstream reduction in emergence rate following adult removal is also unlikely to have contributed towards vector density reduction.

Future experiments may be able to effect a detectable change in vector density by continuing "trapping out" for longer periods, allowing more time for intense trapping to impact upon adult populations and time enough to detect the impact upon subsequent emergence rates.

Larval insecticide applications could also be used to reduce artificially the vector density. Targeting larval stages would be preferable to targeting the adult population as insecticide or attraction to pheromone baits (summarised for other vector species in van Emden and Service (2004)) could potentially confound adult host choice due to repellent and excito-repellent effects of insecticide upon the adults, or disruption of natural preference behaviour. However, selecting and applying an appropriate larvicide may be difficult, largely because relatively little is known about the niches of immature sandfly stages *in situ* (Feliciangeli, 2004), and they are likely to be widely dispersed making targeted application a significant challenge.

Alternatively, commencing "trapping out" in lower vector density environments and including a wider trapping area (not just a single house) could also improve success, if logistically feasible. Nonetheless, experiments such as this are critical to understanding how populations of vectors are controlled and vary over time and space. The implications of not succeeding in reducing local vector populations by such a trapping effort with respect to control interventions are discussed in a later chapter.

In contrast to "trapping out", observation of vector density from November 2011 to July 2012 confirms the presence of natural variation in (mean) sandfly numbers related to the changing seasons, as has been previously observed in the sandflies of Marajó (Kelly *et al.*, 1996) and many other sandfly populations in Latin America (Galati *et al.*, 2003), and elsewhere (Zeledon *et al.*, 1984). Results also indicate possible vector density-dependent changes in host preference.

Increased larval mortality and reduced adult mobility due to high wind and rainfall, including the flooding of larval habitats and oviposition sites, and decreasing temperature may be the factors driving seasonal variation in local population size, as has been seen in the Old World (Dinesh *et al.*, 2001; Galati *et al.*, 2003). Yet, the severity of the 2012 wet season appeared to be less than in previous years (Belém METAR data (Diebel and Norda, 2011)), which may also contribute towards reduced seasonal effects seen during this study, the effect on vectors having commenced later than previously reported. This may be due to the lateness of the rains in Marajó during 2012 where intermittent rains only began in January, compared with December during previous years (Quinnell *et al.*, 1997), and intensity

of the rains was less than previous years (Belém METAR data (Diebel and Norda, 2011)). Yet, as this investigation covered seven months and only subjective measures of rainfall intensity were implemented throughout, and rainfall was only measured from the end of February with no temperature data recorded, it is not within the scope of this study to investigate the magnitude of seasonality between years.

With a background of seasonality in vector density, the over-riding observation from these data is that the number of sandflies caught varies considerably from night to night. The differences between houses, and seasonal variation are both considerably less than the nightly variation. In terms of the transmission dynamics of *Leishmania*, this means that every house has approximately the same average experience, i.e. some nights of very dense vector activity and some nights of very low vector density. The pattern in host preference underlying this heterogeneity, however, does appear to be nonlinear and is consistent within houses over time, as demonstrated by the retention of the nonlinear pattern when averaged monthly data was calculated for each household.

Activity across households within a single night, however, does not appear to average to fit the underlying, and apparently nonlinear, trend in host preference. Here, households differ in their vector density, and therefore preference on a given night, which does not appear to average to a general trend. This may be because host densities also exert an important influence on vector preference (Chapter 6) and have not been taken into account. Instead host density is subsumed into household effects, and preference is calculated across households without taking into account the basis of household differences. Consequently, patterns are lost. The effects of season,

vector density and host density upon preference are all explored quantitatively in Chapter 6, where the effect of host density is explicitly investigated.

Seasonality was associated with shifts in preference, potentially due to density dependent effects. Morrison *et al.* (1995a) also identified changes in sandfly host choice between cattle and pigs in pens, in association with seasonality. This was hypothesised to be due to changes in flight behaviour and aggregation pheromone dispersal in wet weather, plus the beneficial characteristics of sheltered collection sites in the rain. However, it may also represent evidence for density-dependent host preference.

Pheromone-based aggregation behaviour of *Lu. longipalpis* (Dye *et al.*, 1991; Kelly and Dye, 1997), as hypothesised in Chapter 2, is a likely biological explanation for sandfly density-dependent preference. High levels of variability in vector density and preference between nights, occurring separately in households implies that aggregation dynamics are localised and begin anew every night from a different starting point with respect to vector density (Kelly *et al.*, 1997), as is consistent with understanding of how lek-like aggregations form (Jones and Quinnell, 2002).

Seasonal variation of sandfly density has been observed in other parts of Brazil and has been linked to variation in the number of human clinical cases (Resende *et al.*, 2006; Ximenes *et al.*, 2006). On Marajó, reduction in the transmission rate to dogs during the wettest months has also been reported (Quinnell *et al.*, 1997). These findings are likely to be associated with seasonal reduction in fly density and preference for dogs as indicated here, contrary to possible increases in preference to

dogs with decreasing vector density. This apparent contradiction may be attributable to the high level of variability and potentially complex nonlinearities in host choice. These nonlinearities imply that the start point (i.e. the maximum vector population density) would be important to density-dependent effects over seasonal decline. The possible implications of host preference patterns for the transmission dynamics of *Leishmania* are explored in Chapter 6.

4.6. Conclusions

There is considerable variation in sandfly densities between households and nights. The proportion of flies caught associated with different hosts varies with vector density; such that the proportion of flies caught on chickens initially increases with higher vector densities. Overall, there appears to be important nonlinearity in vector biting preference related to vector density. The patterns are retained when sandfly catches are averaged over nights, but are lost when averaged over household. This suggests that any host preference-density relationships are preserved over time, but not with space, i.e. they are created by the immediate local density of sandflies, and not the general density. If localised vector distribution can be manipulated by vector density to draw sandflies away from susceptible hosts, this could play a part in leishmaniasis control, however, it is possible that other determinants of vector preference could be manipulated to elicit the same shift if host preference, such as through the manipulation of host density.

Chapter 5: **Host density-dependent biting preference in sandflies.**

5.1. Introduction

In this chapter, the effect of host density on vector biting rates is considered. In particular, the population of alternative (non-canine, non-human) hosts is manipulated to investigate host density-dependent preference and as initial evidence for the potential zooprophylactic control of zoonotic VL.

Chickens occupy a controversial position with respect to zoonotic VL transmission, as summarized by Alexander *et al.* (2002). In brief, they are a highly abundant blood source, being the most common form of livestock kept in low income households (Alexander *et al.*, 2002), with many households in both urban (Lainson, 1989), and rural settings (Quinnell and Dye, 1994a) being involved in chicken ownership. If availability of blood meals is a limiting factor for a sandfly population, then it follows that an increase in chicken density could increase the carrying capacity of the environment. The addition of hosts may also attract sandflies from beyond the local area due increases in host biomass and associated attractiveness (Quinnell *et al.*, 1992). Plus, in some instances blood source can influence fecundity, with *Lu. ovalesi* being most fecund on chicken blood (Noguera *et al.*, 2006). However, *Lu. longipalpis* do not appear to be more fecund per full bloodmeal upon chickens than dogs (Sant'Anna *et al.*, 2010), therefore preference for chickens over dogs does not, in itself, present a risk of increasing vector density. *Lu. longipalpis* fecundity on humans however, is significantly less (Sant'Anna *et al.*, 2010), as it is for other possible vectors of VL such as *Lu. cruzi* (Chagas *et al.*, 2007); therefore, feeding

upon humans may affect the population carrying capacity. Overall, the relationship between host density and population size is complex, and host density change does not necessarily effect changes in overall vector density (Morrison *et al.*, 1995a).

Chickens are also a well-documented site of *Lu. longipalpis* reproductive behaviour providing a readily available site for lekking behaviour (Jones and Quinnell, 2002), especially when confined to chicken sheds, which appears to be a preferred micro-habitat (Quinnell *et al.*, 1992). The presence of chickens may also encourage closer contact between sylvatic *Leishmania* hosts, dogs and peridomestic sandfly populations. Wild canids may sleep in close proximity to chicken sheds (Courtenay *et al.*, 1994; MacDonald and Courtenay, 1996) or attempt to predate upon chickens (Alexander *et al.*, 2002), and dog ownership co-occurs with chicken ownership as a predation and theft deterrent (Lainson, 1989). Finally, chickens are resistant to infection, therefore it has been suggested that a blood meal of avian blood could also negatively influence parasite development in infected sandflies (Schlein *et al.*, 1983), which would have important implications for host preference and transmission. However, more recent evidence demonstrates no negative effect of chicken blood on the progression of *Leishmania* development and survival within the gut of the sandfly (Sant'Anna *et al.*, 2010). Consequently, chickens (and other non-permissive hosts) potentially act as non-infectious sink for sandfly biting, giving rise to their potential as a zooprophylactic agent of control. The balance between these various factors will determine the extent to which chickens have a negative or positive effect upon transmission.

Evidence from mathematical modelling and analyses of existing and current data (Chapters 2 and 4) indicate that vector biting preference may change nonlinearly with vector density. The key model prediction is that as vector density increases, the relative preference for alternative hosts, such as chickens, is increased compared to dogs and humans, despite a possible absolute increase on all hosts types. These findings have potentially important implications for understanding transmission and implementing control. In particular, these results raise the question of whether vector density and preference could be manipulated through the availability of alternative hosts. Specifically, relative preference could be pushed onto chickens in order to influence transmission by reducing biting density on infectious and susceptible host species. The results of this will be discussed with particular reference to implications for control.

5.2. Aims

The wider aim of this chapter (and Chapter 6) is to investigate the role of chickens in the epidemiology of leishmaniasis through the examination of the relationship between host density and vector density and host preference.

1. To do this the following specific objectives were addressed:
2. Identify the relationship between host density and vector density, via the manipulation of household chicken densities.
3. Investigate the relationship are between host density and sandfly host choice, via the manipulation of household chicken densities.

4. Confirm if the above relationships apply to areas of high, medium and low vector density by repeating host manipulations in regions of high medium and low density of sandflies.

5.3. Methodology

5.3.1. Study Area

All experiments were carried out in the village of Boa Vista (BV), a large village in the Salvaterra district of Marajó Island, Pará State, in northern Brazil. BV was selected as the site for host density manipulations following the successful capture of high sandfly densities, as reported in Chapter 4.

All consenting households within the village which met the selection criteria were enrolled for the host manipulation study. The criteria were that the household was inhabited by both people and an adult dog, but did not have any chickens. Due to the rarity of these criteria only four households were successfully enrolled.

Experimental chicken sheds were constructed following the typical local design, with walls constructed of regularly spaced wooden sticks (approx. 2cm spaced) and the roof made of dried palm leaves. Experimental sheds were 1m wide, 1m deep and 1.5m high and contained 3 roosts. However, an existing but disused shed of wider proportions (approx. 2m wide) was utilised in house K (see Figure 4.1).

5.3.2. Collection Protocol

As previously described in Chapters 3 and 4, traps were set in three domestic locations in close association with each host of interest in order to sample sandfly host preference. One standard CDCLT was placed in the bedroom of the house, one in the chicken shed and a hood modified CDCLT was used upon a caged dog. Trap position was optimised for each individual trap location, with the trap entrance being positioned as close to the host as possible. Traps were set equidistant from one another; ensuring dogs were caged at a distance from the house and chicken shed equal to the distance between the house and shed. This was aimed to remove distance as a confounder of host choice and reduce dog proximity to other potential confounders such as lights and domestic facilities.

Traps were set by 6.30pm and collected at 6.30am, with CDCLT bags being changed at 9.15pm as part of the investigation into sandfly activity throughout the night (described in Chapter 4). All closed CDCLT bags were left hung next to the CDCLT so that natural pheromone production and associated sandfly recruitment to the trap site was not interrupted by sandfly removal.

Upon collection, flies from each catch were transferred to ethanol, whereupon the dead sandflies were separated by sex and bloodmeal status before being counted under dissection microscope.

5.3.3. Manipulation of chicken host density

To investigate the effect of alternative host density upon fly numbers and distribution between hosts in the peridomestic environment experimental chicken sheds were constructed as described above on the premises of four households, not normally harbouring chickens.

Baseline sandfly density upon each host of interest was estimated for each household by trapping at all three domestic locations for a period of three nights prior to the commencement of host manipulations. Pairs of chickens were then added to each experimental shed and the density of flies upon each host type sampled three nights later. Trapping was repeated the following night if there had been rain or strong wind, as these abiotic factors were expected to interfere with capture on these nights (Ximenes *et al.*, 1999) (also see Chapter 3). Sampling was carried out after a three night lag time in order to permit sandfly "discovery" of the new hosts (Jones and Quinnell, 2002). Chickens roosted in the experimental sheds at night but were allowed to roam during the day, except when there were issues of escape or predation. Pairs of chickens were incrementally added and the associated sandfly densities sampled in this way until 20 chickens had been introduced to all sheds and the distribution of sandflies measured across all three household locations. After this maximum was reached, all chickens were removed, returning households to pre-intervention host densities. Vector density and distribution between hosts continued to be monitored for the next five nights in order observe the effect of host removal upon sandfly density and preference.

This trapping regime was repeated in BV three times over the course of the seasons in order to estimate the effect of host manipulation when local vector density was predicted to be high, medium and low, corresponding to the transition between dry and wet seasons from January and June 2012. The first round of sampling was carried out between January 17th and March 25th, the second between April 21st and May 25th and the third from 27th May to July 1st 2012.

The initial protocol of incrementally adding pairs of chickens to sheds was revised after the completion of the first trapping round, due to the constraints of time. For the second and third experimental round three chickens were added upon each host addition, to a revised maximum of 21 chickens, and trapping was no-longer repeated if there had been rain in response to the increased frequency of rainy nights. Together these shortened the experiment duration in the latter rounds.

5.3.4. Analysis

In order to investigate the relationships between host density, vector density and biting preference, graphs are drawn in the first instance and basic analysis performed where appropriate using linear regression. More complex analyses follow in Chapter 6. As before, absolute fly count data was transformed by natural logarithm to approximate a normal distribution (see Appendix E), and all data presented in STATA v.11 (StataCorp LP).

Due to the potential delay in recruitment of sandflies when hosts were added, and possible residual attractant effects once they were removed, analysis of chicken

numbers was done using an estimate of effective chicken number. Effective chicken density (*ECD*) was calculated as the weighted sum of the number of chickens (*C*) present within the chicken shed at the time of trapping (*t*), and over the previous five nights.

$$ECD = C_t + \kappa C_{t-1} + \kappa^2 C_{t-2} + \kappa^3 C_{t-3} + \kappa^4 C_{t-4} + \kappa^5 C_{t-5} \qquad (5.1)$$

The contribution to the effective chicken density made by each previous night was determined by a diminishing function (κ) for each of the previous five nights (*t-n*) (Table 5.1).

Table 5.1: The weighting of night's chicken density contribution to ECD when κ=0.8.

Night	*κ*	*Weighting*
t		1.00
t-1	κ	0.80
t-2	κ^2	0.64
t-3	κ^3	0.51
t-4	κ^4	0.41
t-5	κ^5	0.33

5.4. Results

5.4.1. Data summary

In order to investigate the effect of host density upon biting preference, the number of chickens was manipulated in four households over the course of 6 months. Host density was increased from zero to a maximum of 21 chickens in each trapping

round. However as indicated in Figure 5.2, there were frequently fewer chickens per household than the desired number, highlighting the issue of loss of chickens from households, especially during the first round. However, in general the expected host density was achieved. Figure 5.1 also shows that no host manipulation or trapping was carried out in house A during the second trapping round. Trapping was interrupted here due to the death of the household dog, but recommenced for the third round following the householder's purchase of a new animal.

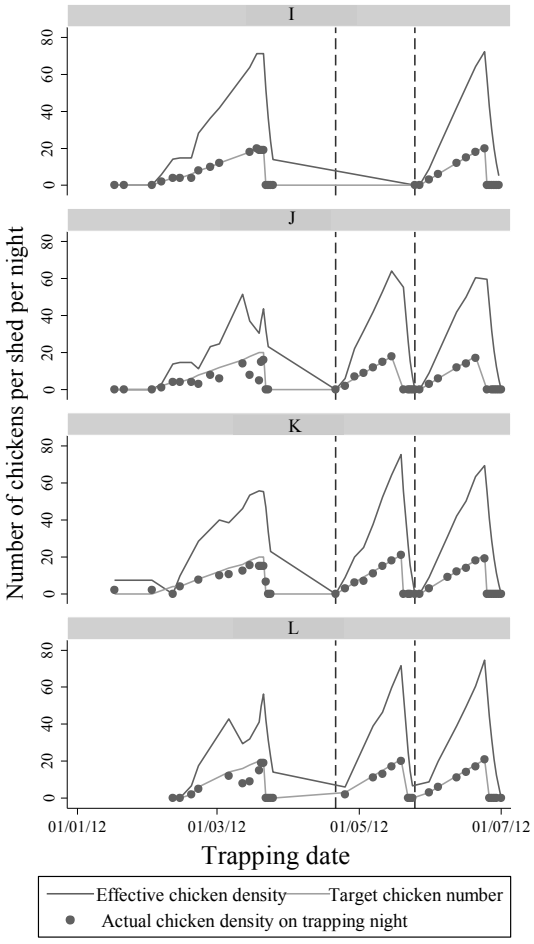


Figure 5.1: Summary of the actual, planned and weighted effective chicken densities (when κ=0.8) in each household over the course of the host manipulation experiment. Dashed lines delineate between experimental trapping rounds.

In order to estimate the lag and residual effects of chicken numbers on sandfly captures, Figure 5.2 shows the decrease in sandfly numbers after removal of all chickens. This confirms that it takes time for sandfly numbers to decline following host manipulation, particularly on chickens, where the effect of time after intervention removal is significant (*β=-0.30, T=-3.53, P=0.001*). This plot also indicates that the majority of fly density reduction occurs within five nights, therefore five nights was assumed to be a suitable lag time over which to take chicken numbers into account and calculate the effective chicken density used henceforth.

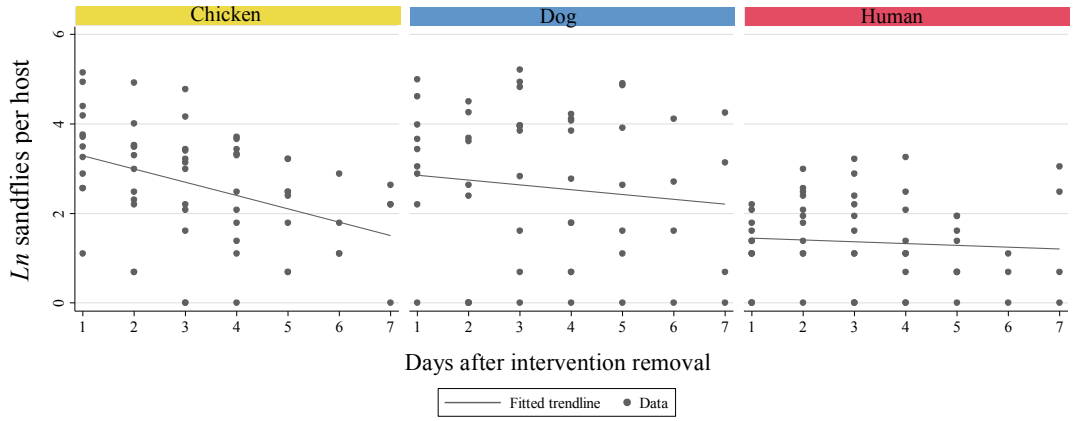


Figure 5.2: Sandfly density per host in the nights following the removal of all chickens on the morning of day 1. Note: there are approximately 20 chickens per shed at night 0. Night 1 represents the first night with zero chickens. Host densities at the human and dog traps are constant throughout.

5.4.2. Effect of host density on vector density

Host manipulations occurred over a period of months, therefore the effects of seasonality seasonality has a significant effect on the baseline sandfly density per host and household between trapping rounds (Figures 5.3a-d). This confirms the seasonal trend identified in Chapter 4 which is associated with change in both vector

density and preference throughout the experiments. In order to investigate host preference in relation to host densities, rather than including seasonality as a continuous time variable in these analyses, trapping round was treated separately so that long-term seasonal trends do not significantly influence the outcomes of the analysis. Therefore, trapping round is treated as a fixed effect in later analyses (Chapter 6).

In addition to seasonally driven changes in vector density, variation in the total number of sandflies caught per household over the manipulation series also appears to reflect changes in effective chicken density (Figure 5.3a). This is particularly evident following the removal of chickens towards the end of each experimental round, when there are multiple consecutive nights of trapping. Host specific sandfly catch rates also show variation in association with effective chicken density over the experiments, particularly during trapping round 1 (Figures 5.3b-d). The number of flies on dogs shows a marked reduction with effective chicken number, followed by an increase upon host removal. The reverse is true on chickens, where increasing effective chicken number through time is associated with peaks in sandfly numbers. This likely reflects changes in preference for each host following the introduction of chickens rather than changes in overall vector density, a hypothesis which is explored below.

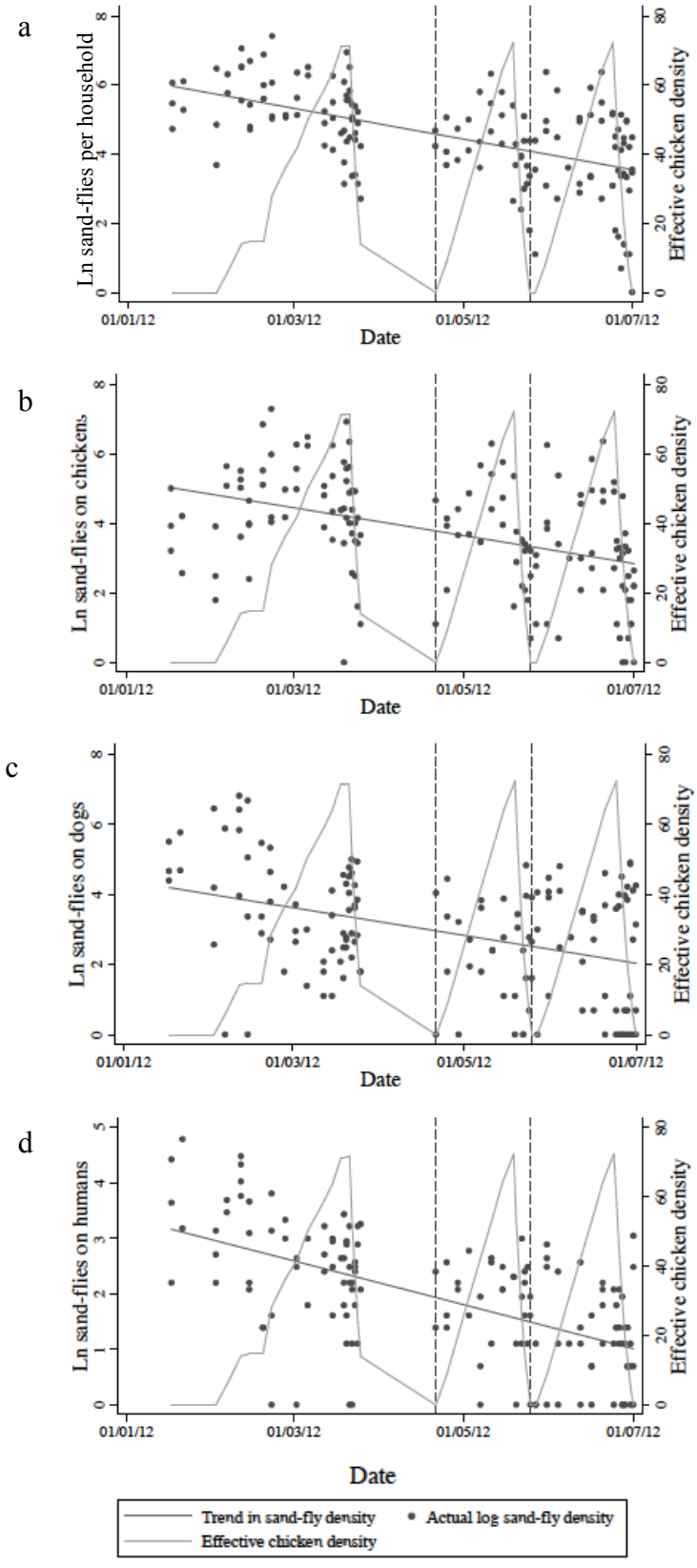


Figure 5.3 a-d: Nightly *ln* sandfly numbers caught (a) across all household trapping locations, (b) on chickens, (c) dogs and (d) humans over the course of the host manipulations. Change in the effective chicken density highlighted in pale grey and the general decline in vector number in dark grey. The transition between trapping rounds is demarcated by dashed black lines.

As shown in Figure 5.4, the relationship between overall household vector density (summed across all hosts) and effective chicken density across the three trapping rounds failed to reach statistical significance. However, it may be possible to see a slight reduction in fly numbers with host density during round 1 (*β=-0.01, T=-2.29, P=0.026*); this may relate to seasonal effects rather than a negative sandfly and host density relationship.

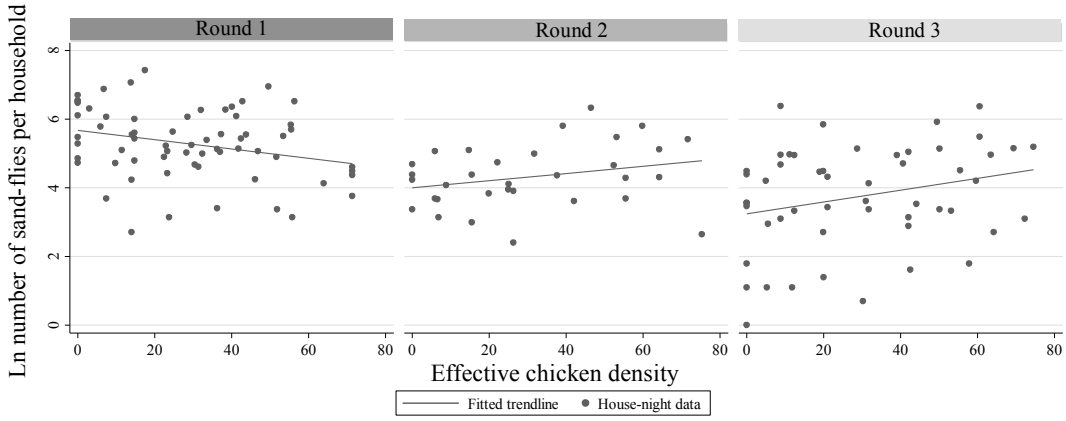


Figure 5.4: Change in overall *ln* household vector density associated with manipulation of the effective chicken density during three trapping rounds.

In spite of the minor impact of chicken density upon overall vector density there is clear change in the number of sandflies per host over the course of the host manipulations (Figure 5.5). Specifically, once round has been included as a fixed effect, regression confirms that the number of sandflies on chickens significantly increases with chicken density (*β=0.02, T=3.76, P<0.0001*), while decreasing upon dogs (*β=-0.02, T=-2.99, P=0.003*). On humans however, there appears to be no significant trend (*β=-0.005, T=-1.41, P=NS*), until an interaction term between effective chicken density and round is included (*β=-0.02, T=-3.08, P=0.002*). This highlights a negative association between the number of flies caught on humans and effective chicken density, but also that this trend may differ between rounds. The

rounds differ from one another in their baseline vector density, which is lower from one round to the next due to seasonaity. These trends in sandfly density per host are most distinct in trapping round 1 (Figure 5.5), where the local vector density is initially higher, and changes in vector density per host with effective chicken density appear to become less marked with each trapping round. This indicates possible interaction between seasonality and host density per host, and that the effect of host manipulation is reduced at lower vector densities, particularly on humans.

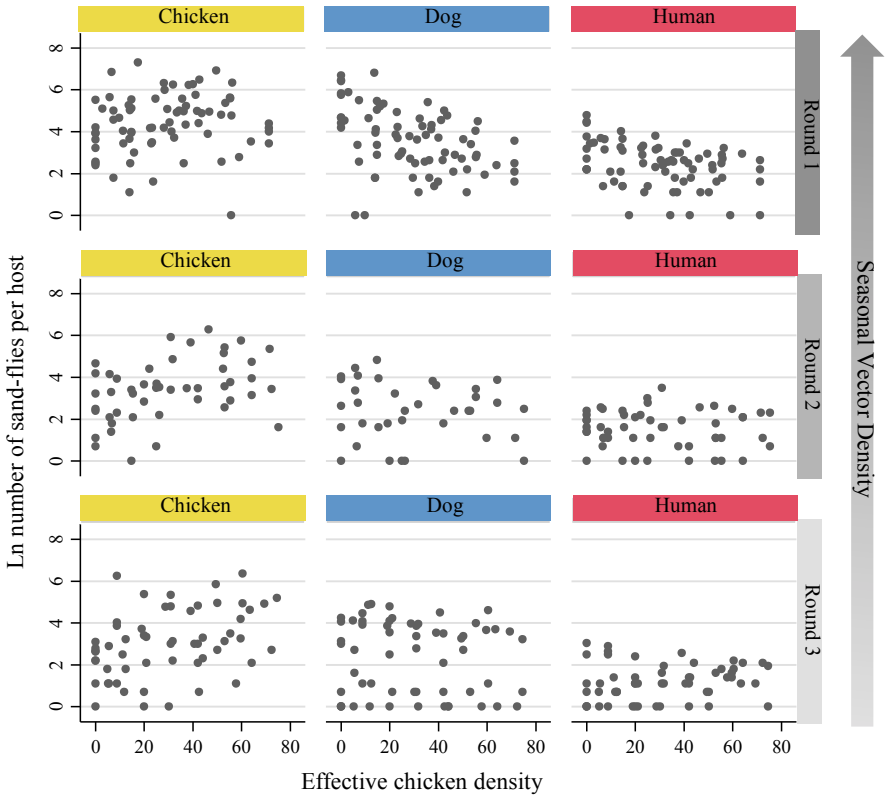


Figure 5.5: Change in the log number of sandflies per host with effective chicken density during each trapping round.

5.4.3. Effect of host density on vector preference

Change in absolute number of flies on each host is also mirrored in the changing proportions of sandflies on each host with effective chicken density (Figure 5.6). The preference for chickens appears to increase with effective chicken density, whilst reducing upon dogs. The proportion caught upon humans remains relatively stable throughout the host density manipulations. These relationships, however, are not clearly defined, and again may become less marked in the later trapping round, possibly as a result of manipulating in low local vector density environments.

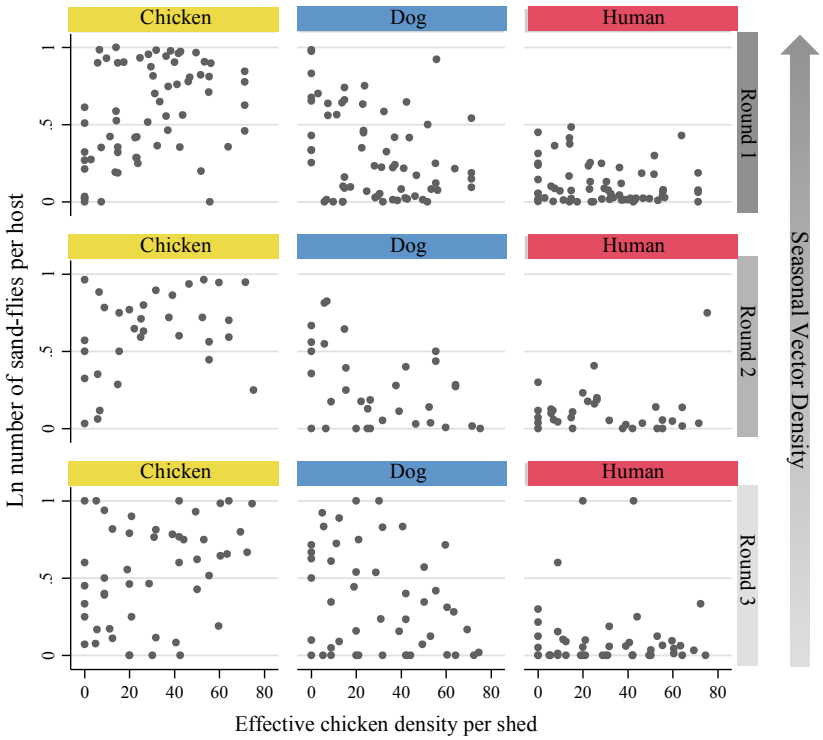


Figure 5.6: Change in the proportion of total sandflies ("preference") captured on each host with changes in effective chicken density during the three trapping rounds.

5.4.4. Effect of host density on both vector density and preference

Given that natural variation in vector density (Chapter 4) and host density may both affect host preference, it is important to consider these two effects in tandem. In order to do this. effective chicken density was arbitrarily divided into four groups, representing nights with high, medium, low and zero effective chicken density.

Figures 5.7-5.10, below, demonstrate that vector density may be associated with host preference, as hypothesised in Chapter 2, but moreover that the association differs depending on the effective number of chickens in the chicken shed. For example, the proportion of flies on chickens increases with vector density and decreases on dogs and humans across all trapping rounds (similar to that presented in Figures 2.4, 2.5), but only when effective chicken number is high (here shown above 50 (Figure 5.7). Preference becomes more variable at low effective host densities, and the strength of these relationships begins to wane when effective chicken number is low (<50) (Figure 5.8-5.10). The vector density-preference relationship appears particularly weak on nights with zero effective chickens (zero chickens in the shed for the past 5 nights), yet this may be due in part to the low number of nights where zero effective chickens were available.

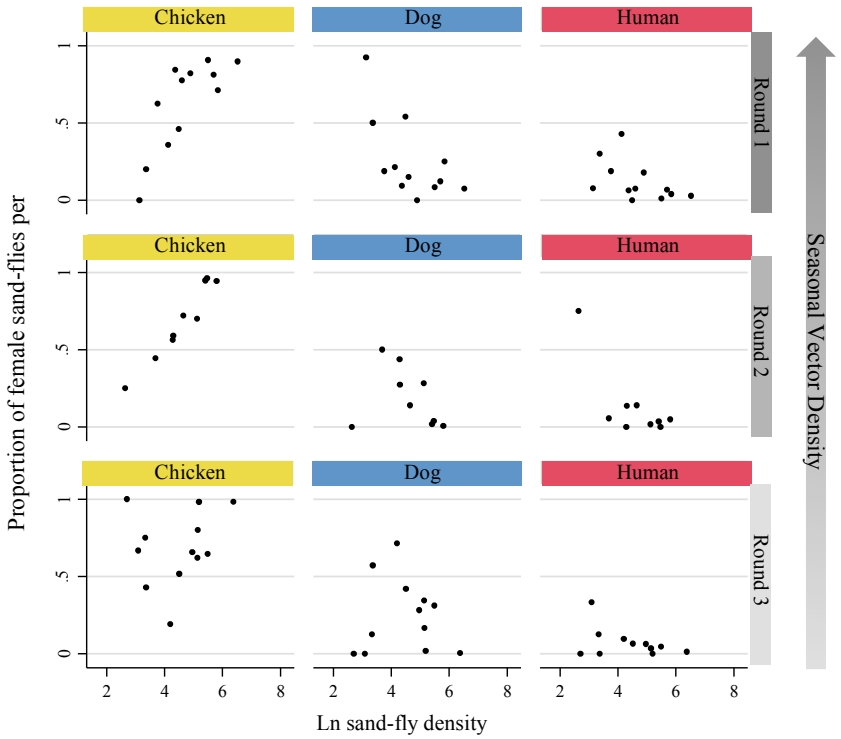


Figure 5.7a: Change in the proportional preference of female sandflies for each host with vector density during each trapping round on nights with an effective chicken density >50.

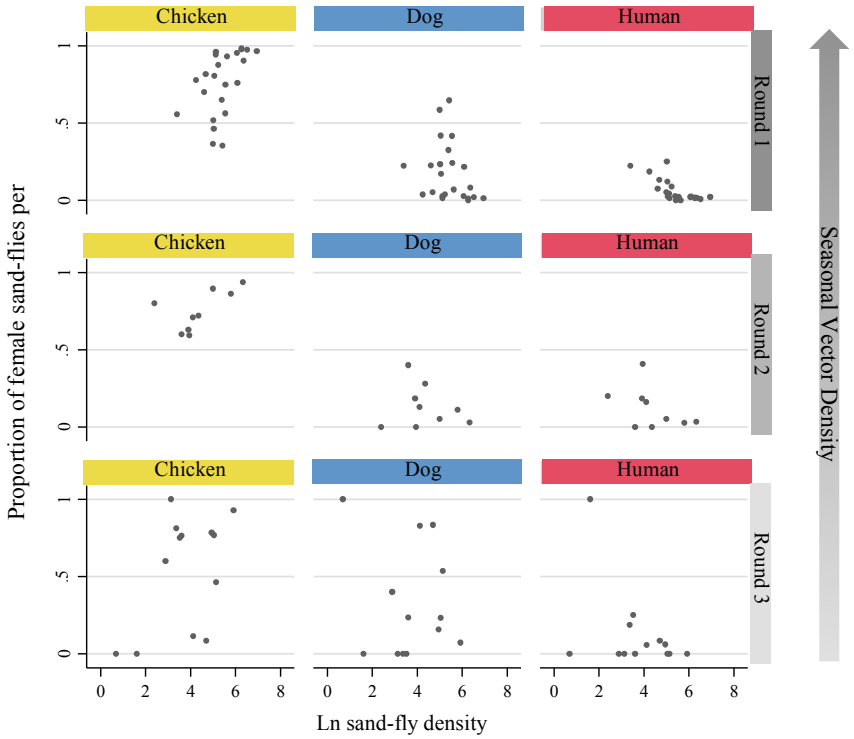


Figure 5.8: Change in the proportional preference of female sandflies for each host with vector density during each trapping round on nights with an effective chicken density 25-49.

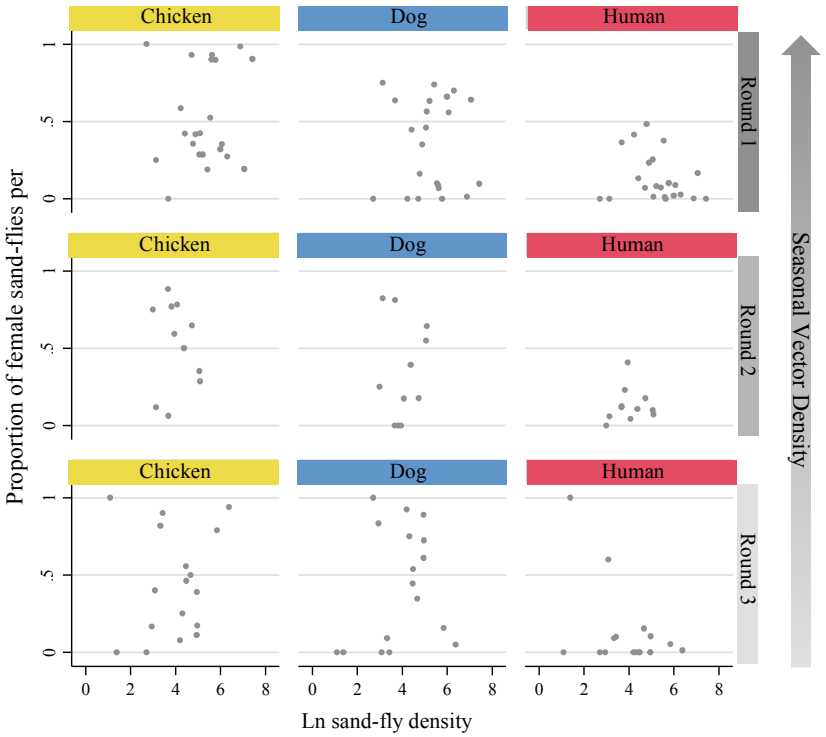


Figure 5.9: Change in the proportional preference of female sandflies for each host with vector density during each trapping round on nights with an effective chicken density 1-24.

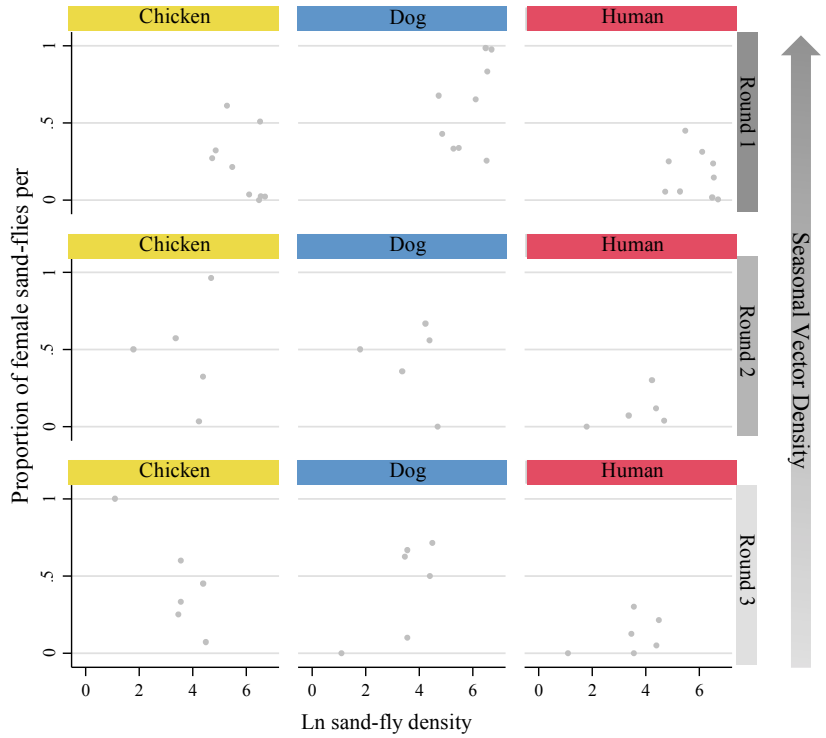


Figure 5.10: Change in the proportional preference of female sandflies for each host with vector density during each trapping round on nights with an effective chicken density of zero.

5.5. Discussion

This chapter presents results of numbers of sandflies and biting preferences over a period of months during which chicken density is experimentally manipulated. Overall the results highlight a significant underlying relationship between host density and sandfly preference, despite the highly variable nature of sandfly host choice between nights. New animal sheds were readily colonised following the addition of chickens, which influenced the local distribution of sandflies between key hosts.

As previously suggested by Alexander *et al.* (2002) and Lainson and Rangel (2005), the keeping of animals, namely chickens, and their sheds may promote sandfly activity in the peridomestic environment. The availability of peridomestic hosts has been associated with the domestication of other sandfly vectors, such as *Lu. whitmani*, demonstrated via both behavioural and molecular studies (Ishikawa *et al.*, 1999; Ready *et al.*, 1998). Here, increased chicken density is shown to translate into increased aggregation upon chicken, rather than increased sandfly abundance across all peridomestic locations in general. In fact, the absolute and relative preference for non-manipulated host types (dogs and humans) may decrease.

This may be is indicative of a more ornthophilic habit in *Lu. longipalpis* than previous reported, but moreover that the preference for chickens influences the preference for other hosts. Such a pattern may arise due to pheromone based aggregation dynamics, whereby males commence lekking in the early evening before females (Jones and Quinnell, 2002; Kelly and Dye, 1997), selecting hosts on the basis of biomass (Quinnell *et al.*, 1992), and accessibility (Quinnell and Dye, 1994b).

Once in position, males produce sex pheromone which works in synergy with host odour to attract females and more males to the location (Bray and Hamilton, 2007b), resulting in an aggregated distribution of sandflies across the peridomestic landscape (Kelly and Dye, 1997; Kelly *et al.*, 1996). It is possible, therefore, that the addition of chickens influences the initial distribution of lek sites, by creating areas of high attractiveness, enhanced by biomass contributions. Chickens are less active at the onset of sandfly blood feeding, hence may be less defensive and lessen the dispersion to leks on dogs.

Sandfly abundance is also likely to influence fly distribution (as hypothesised in Chapter 2 and 4), whereby higher fly densities lead to greater pheromone production and concentration of activity on hosts where lekking has commenced. Contrary to Chapter 4, the influence of vector density upon host choice is more evident here, being most prominent at high effective chicken densities. It may be that the presence of high alternative host numbers stabilises the complex and highly variable vector density-preference relationships. Making alternative lek sites more readily available and attractive for when the excessive aggregating effect of pheromone production does occur on high-density nights.

Local vector density did not appear to increase with host density. However, the effect of seasonal decline is a significant driver behind vector density variation (Chapter 4) (Zeledon *et al.*, 1984). This effect may limit the extent to which host-dependent increases in vector density can be observed over the experimental time course. Reductions in vector density associated with wind and rain (Ximenes *et al.*,

1999; Zeledon *et al.*, 1984) may mask increases in local vector density due to the addition of hosts.

Additionally, the time scale of the current investigation may not have been sufficient to allow for complete recruitment of sandflies to the experimental households, or been long enough to observe downstream effects on vector population sizes. Yet, vector population size is only likely to be influenced by host density if bloodmeal availability is a population limiting factor, or changes in host preference lead to significant changes in population reproductive success. Egg production on human blood appears to be significantly lower than on other peridomestic sources of interest (Sant'Anna *et al.*, 2010), but as the majority of flies are caught on dogs or chickens, the small change in preference for humans is unlikely to exert a large effect. As such Morrison *et al.* (1995b) identified that changes in host density do not necessarily result in changes to vector density. By contrast, others have found vector abundance to be associated with the presence of hosts such as chickens (Fernandez *et al.*, 2010). However, when the capture methodology is aimed at sampling the "worst scenario" household location, as by Fernandez *et al.* (2010), comparing densities between households may not reflect overall differences in vector abundance, but in the level of aggregation at chicken sheds, which is relatively high (Brazil *et al.*, 1991). This is likely to result in the overestimation of the association between chickens and sheds and local vector density. The protocol suggested here, sampling across multiple domestic sites, may give a more reliable estimation of local sandfly density from which to draw conclusions, yet an extended protocol would be required to assess long-term population level effects of host manipulation.

One possible implication of these results is that in areas of high vector density the addition of chickens could divert flies from infectious hosts, a possibility which is explored in greater detail in Chapter 6. However, if host availability is a significant limiting factor for the vector population, then addition of chickens could increase the total density of vectors.

It should also be noted that diversion of flies away from dogs and humans in association with alternative host numbers may be dependent on there being sufficient distance between the night-time locations of the respective hosts. Observations in the field confirm that canine sleeping locations do not usually overlap with chickens in sheds, but if they did, promoting aggregation to chicken sheds through host manipulation may also increase sandfly activity upon dogs.

In some Brazilian states, such as São Paulo (SP), the local health authority discourages the keeping of chickens, in an attempt to limit noise and smell associated with these animals (Orin Courtenay, pers. comm.). Yet, chicken ownership is highly advantageous to householders in other ways, such as the provision of eggs and meat for both consumption and sale. The benefits of keeping hen houses in terms of nutrition, food security and income, especially among poor populations, are well documented (Moreki *et al.*, 2010). When combined with the benefits of chicken density in manipulating sandfly-biting preference it becomes apparent that the benefits of chicken ownership may outweigh the negative aspects associated with noise and pollution. Local vector ecology in SP, however, is different to that of Marajó (Young and Duncan, 1994), with relatively lower sandfly abundance, being semi-urbanised, and exhibiting different seasonality and habitat conditions. It is possible therefore that the above results will not directly translate, especially if

sandfly numbers are limited by host availability. Together, this indicates that the role of chickens warrants further investigation across a range of epidemiological situations.

One of the main problems encountered during data collection was that of inconsistent chicken densities, whereby not all the experimentally introduced chickens were present within the shed on the trapping night. This was normally due to chickens roosting elsewhere, a common occurrence as chickens were new to the households, however, instances of robbery and predation also contributed. In order to combat this, chickens were located and returned to the shed where possible, and housed within chicken sheds both day and night when problems continued. To control for this source of variation within the data, and due to the lagged response time or cumulative effect of chicken density on vector numbers, effective chicken density was calculated. Here, it was assumed that the number of chickens on a trapping night is consistent with the number on preceding (unobserved) nights, rather than the expected chicken number. Nevertheless, variation in the number of chickens roosting in experimental sheds on nights proceeding the trapping night (i.e. unrecorded nights), may be a source of inaccuracy in the calculation of effective chicken number, which was not possible to check within the protocol. Future experiments should, however, incorporate a step for the confirmation of chicken densities on all nights in order to remove this possible source of inaccuracy.

Future work should focus upon generational effects of host numbers and composition upon sandfly density. Furthermore, in order to remove the confounding relationship between host density and vector density, which appear to interact in

their effect upon host choice, the experiment could be repeated in a closed environment so that basic host choice experiments using fixed numbers of flies could be used. Alternatively, repeating the above experiments in different environments, where hosts are scarcer or micro-climates for fly egg laying are different, as the starting point for host and vector density could lead to very different results and therefore interpretation with respect to control.

5.6. Conclusions

Overall, it appears that chicken density and fly density may combine to influence the absolute and relative preference of sandflies for different hosts over the seasons, with potentially far reaching implications for control. However, in depth analysis of these data is required to elucidate the nature of these relationships with respect to transmission.

Chapter 6: **The effect of host density manipulations on sandfly preference and force of infection.**

6.1. Introduction

As basic transmission models show, for the successful transmission of VL to humans both a competent reservoir and vector population must be present, with sufficient biting occurring upon both dogs and humans to maintain the disease (Dye, 1996). It follows therefore that the dynamics of vector host choice are vital to maintaining transmission and determining the risks to humans. There is expected to be a maximum of transmission risk to humans: if all flies feed on dogs or all feed on humans there will be zero risk of transmission to humans, but between these two extremes of host preference there must be a maximum level of risk to humans.

As presented in Chapters 4 and 5, data from small studies visually indicate that underneath substantial nightly variation in vector density, sandfly preference and distribution between peridomestic hosts may be influenced by both host and vector densities. Specifically, manipulation of chicken density and observation of sandflies over seasonal decline in vector density resulted in reduced preference for dogs and humans with increasing chicken host and vector density. It is therefore hypothesised that host choice in *Lu. longipalpis* is dependent on the densities of both flies and accessible hosts, which would have important implications for understanding VL dynamics and for design of intervention programmes.

6.2. Aims

The aim here is to investigate host and vector density-dependent host choice in sandflies via statistical modelling of the data described in Chapter 5, to establish firmly the existence of any density-dependent effects upon sandfly feeding behaviour, and on proxy measures of transmission risk to dogs and humans.

1. To do this the following objectives must be met:
2. Analyse the relationship between chicken host density and the number of sandflies caught upon humans and dogs.
3. Analyse the association between chicken host density and the proportion of sandflies caught upon humans and dogs.
4. Analyse the effect of vector density upon the proportional preference of sandflies for humans and dogs.
5. Investigate how vector density dependence and host density dependence interact to effect biting preference.
6. Analyse the relationship between chicken host and vector density and proxy measures of the force of infection (FOI) to humans and dogs.

6.3. Methodology

6.3.1. Data collection

Data collection is outlined in Chapter 5. In brief, the number of chickens per shed was manipulated from zero to a maximum of 21 in four households over the course of 3 trapping rounds, in a leishmaniasis endemic region of Brazil. Over the course of

host density manipulations the number of sandflies visiting humans, chicken sheds and dogs were monitored by standard CDCLT and modified CDCLT on dogs. Together these three measures were assumed to represent localized household vector density at each house per night.

6.3.2. Analysis

A number of outcome variables were considered important for this investigation. Direct outcomes of interest were the absolute and relative sandfly density on humans and dogs. Additional outcomes of a proxy for FOI on dog and humans were also calculated as outcomes of interest.

The FOI is the incidence rate (rate at which new infections arise) per susceptible host, and is a key epidemiological process which indicates the infection transmission potential to particular hosts. FOI is important in the calculation of transmission rates once host infection statuses and the probability of transmission events being successful are known (see Chapter 2). Here, FOI is assumed to be proportional (~) to the number of sandflies visiting humans ($\theta_H.V$) or dogs ($\theta_D.V$) which may be been infected on a previous feed.

$$FOI_H \sim \theta_D \theta_H V_S \quad (6.1)$$

$$FOI_D \sim \theta_D^2 V_S \quad (6.2)$$

The proportion of sandflies which visited reservoir hosts, dogs (θ_D), on a given night is assumed to represent the proportion of sandflies which has been exposed to infection. Logically, transmission events from dogs to humans will not occur within a single night, but given that chicken density varied between capture nights, within night dynamics were used as a proxy for sandfly distributions under given host densities. This is supported by the results of Chapter 4, where the vector density-preference relationship was preserved after averaging over time.

All data were analysed using STATA v11.0 (StataCorp LP) using mixed models with two hierarchical levels due to structuring within the data, whereby all nightly captures were nested within household ID (Rabe-Hesketh and Skrondal, 2012). All sandfly count data were transformed by natural logarithm [$ln(n+1)$] in order to approximate a normal distribution and analysed using linear mixed models. Proportional outcomes were analysed using generalised linear mixed models (GLMM) with the logit link function and binomial error distribution (Rabe-Hesketh *et al.*, 2004). Finally, FOI data were also natural log transformed, so as to avoid negative values from linear models, but the data remained overdispersed despite the transformation. Therefore these data were also analysed using GLMMs, specifying a negative binomial distribution and log link function in addition to random effects. FOI analysis was carried out using the *runmlwin* (MLwiN v2.26) extension in STATA v11.0 (Rasbash *et al.*, 2012).

As previously demonstrated (Chapter 4), seasonality has a negative effect upon vector density over the period of this investigation. In order to account for this source of variation and differences in experimental regime between trapping rounds,

round was routinely incorporated into all models as a fixed effect. The explanatory variables of interest were effective chicken density and overall vector density. Interaction between these predictors of interest was also explored. However, due to potential issues of collinearity following the generation of interaction terms, continuous variables were grand mean centred for interaction analyses (Aiken and West, 1991). Additionally, effective chicken density was categorised in order to explore nonlinear main effects and interactions. Categorisation involved splitting the variable effective chicken number into five groups with roughly equal sample sizes per category (Table 6.1).

Table 6.1: Frequency of house-nights where effective chicken density fell into the following ranges.

Category	*Frequency*
0-15 chickens	53
16-30 chickens	26
31-45 chickens	31
46-60 chickens	23
>60 chickens	16

6.4. Results

In order to assess the effect of host density upon sandfly density, preference and transmission potential, chicken density per shed was manipulated between 0-21 chickens in four households over a period of seasonal vector decline. This resulted in the capture of over 33,674 sandflies over 148 nights of activity during three rounds of trapping. The geometric mean shows that on average 92.88 flies were caught in each household per night; however, vector density was highly variable (over-

dispersed) with capture density ranging between 0-1,680 flies per household per night.

6.4.1. Vector density on humans and dogs

Total household vector density did not appear to be significantly related to household host density (*β=0.004, z=1.18, P=0.240*) after the significant fixed effect of trapping round and structuring in the data due to repeated measures on households had been taken into account. This indicates that household host and vector density are independent, and therefore the density of sandflies upon specific domestic hosts is unlikely to be confounded by additional local sandfly recruitment *in specific response* to local host density changes.

Absolute vector density upon dogs appears to be statistically significantly and negatively related to effective chicken density (*β=-0.014, z=-2.83, P=0.05*) once the effect of household ID and round have been taken into consideration. This indicates that the number of flies upon dogs reduces as chicken host density increases. Similarly, the density of sandflies upon humans appears to decrease in association with increasing effective chicken density, though this relationship reaches only borderline significance (*β=-0.006, z=-1.80, P=0.071*).

Further inspection of these relationships using a categorical measure of effective chicken density confirms the negative relationship between absolute fly density on dogs and humans with effective chicken number (Table 6.2).

Table 6.2: Linear mixed model analysis of relationship between categorical chicken density and vector density on dogs, taking into account fixed seasonal effects and structuring within the data.

Fixed effects	Category	Density upon humans (ln(n+1)) Co-efficient (95% CI)		Density upon dogs (ln(n+1)) Co-efficient (95% CI)	
	0-15				
Effective	16-30	-0.37	(-0.80-0.08)	-0.58	(-1.23-0.06)
chicken	31-45	-0.44**	(-0.86- -0.03)	-0.74**	(-1.34- -0.13)
density	46-60	-0.42	(-0.87-0.04)	-0.80**	(-1.48- -0.13)
	>60	-0.23	(-0.76-0.29)	-0.70	(1.48-0.07)

Note: **indicates significant at the P≤0.05 level.

However, this also reveals possible nonlinearity in these relationships, as only the mid-range effective chicken densities are associated with a significant reduction in vector density upon either host of interest. Quadratic terms, however, fail to become significant predictors.

6.4.2. Proportional preference for dogs and humans

By comparison, the relationship between the effective chicken density and the *proportional* preference for dogs and humans appears to be significantly nonlinear (Table 6.3). Increases in effective chicken density are initially associated with significant reductions in preference for both dogs and humans, but this negative association lessens at higher host densities to become marginally positive. Henceforth, effective chicken density is included as a categorical variable in order to aid interpretation of nonlinear interaction effects.

Table 6.3: GLMMs of effective chicken density and the proportion of sandflies on humans and dogs, taking into account fixed seasonal effects and data structuring.

Fixed effects	Proportion on humans Co-efficient (95% CI)		Proportion on dogs Co-efficient (95% CI)	
Effective chicken density	-0.03*	(-0.04, -0.02)	-0.11**	(-0.11, -0.10)
Effective chicken density (squared)	0.0002*	(0.0001, 0.0004)	0.001*	(0.0009, 0.0010)

Note: **indicates significant at the P≤0.05 level; *borderline significance at the P≤0.1 level.

Increasing total household vector density also has a significant negative effect upon the proportion of sandflies caught on humans (*β=-0.70, z=-22.51, P<0.0001*) and dogs (*β=-0.19, z=-12.10, P<0.0001*), after round and house ID have been taken into account.

Inclusion of both host and vector density together in a multivariate mixed model confirms the significant negative effect of chicken density upon preference for humans and dogs, with the lowest proportion being caught on these hosts when effective chicken density falls between 46-60 chickens. Yet the full model also reveals significant interaction between host and vector density in association with host preference (Table 6.4).

On humans the interaction effect is strongest at medium-low (31-45) and high (>60) effective chicken densities, with lower coefficients at effective chicken densities of 16-30 and over 60. The lack of a significant interaction between chicken and vector density when effective chicken number is 45-60 but may be indicative of a small

group size, however, the sample sizes per category is approximately equal, implying this is not the case.

Table 6.4: GLMMs to describe the associations between effective chicken density and vector density with the outcome of proportional preference for humans and dogs, taking into account fixed seasonal effects and structuring within the data.

Fixed effects	*Category*	*Proportion on humans* *Co-efficient (95% CI)*		*Proportion on dogs* *Co-efficient (95% CI)*	
Effective chicken density /ECD	0-15				
	16-30	-0.17**	(-0.35, -0.0006)	-0.83**	(-0.95, -0.71)
	31-45	-0.72**	(-0.92, -0.53)	-0.77**	(-0.89, -0.64)
	46-60	-0.80**	(-1.02, -0.58)	-1.46**	(-1.61, -1.32)
	>60	-0.73**	(-0.97, -0.49)	-1.14**	(-1.31, -0.97)
Centred *ln* total vector density/*Ln*Total		-0.67**	(-0.74, -0.59)	0.003	(-0.05, 0.05)
Interaction	*Ln*Total X ECD0-15				
	*Ln*Total X ECD15-30	-0.43**	(-0.58, -0.29)	-0.48**	(-0.56, -0.40)
	*Ln*Total X ECD30-45	-0.25**	(-0.40, -0.09)	-0.94**	(-1.04, -0.85)
	*Ln*Total X ECD45-60	-0.10	(-0.26, -0.06)	-0.84**	(-0.96, -0.73)
	*Ln*Total X ECD>60	-0.48**	(-0.77, -0.20)	-0.87**	(-1.04, -0.70)
Round	1				
	2	-0.70**	(-0.88, -0.53)	-0.76**	(-0.87, -0.65)
	3	-1.25**	(-1.44, -1.07)	-0.16**	(-0.25, -0.07)
Intercept		-1.23**	(-1.35, -1.12)	0.18**	(0.09, 0.26)
Random effects		*Estimate (S.E.)*		*Estimate (S.E.)*	
House ID		0.06	(0.02)	0.08	(0.004)

Note: **indicates significant at the P≤0.05 level.

By contrast, the reduction in preference (coefficient) for dogs with increasing vector density appears to be greatest at mid (≥31-45) to high effective chicken densities (Table 6.4). However, the main effect of vector density becomes non-significant

following the inclusion of statistically significant interaction terms. This indicates that the combined effect of host and vector density is a more important predictor of proportional preference for dogs than vector density in isolation.

6.4.3. Force of infection

The computation of the FOI presented here is assumed to be a proxy for the true force of infection upon dogs and humans (equations 6.1, 6.2), calculated from the density (V) and proportional distribution of flies between hosts (θ_D and θ_H) on a given night. Despite the use of nightly data, the proportion of female flies on dogs (θ_D) and the number of females upon humans (V) are not significantly related to one another (β*=-0.23, z=-0.96, P=NS*), once fixed and random effects have been taken into account. Therefore the FOI on humans can be calculated without problems of dependence in the constituent variables (Figure 6.1). The number (V) and proportion of sandflies on dogs (θ_D), however, are intrinsically related (β*=-2.80, z=10.71, P<0.0001*).

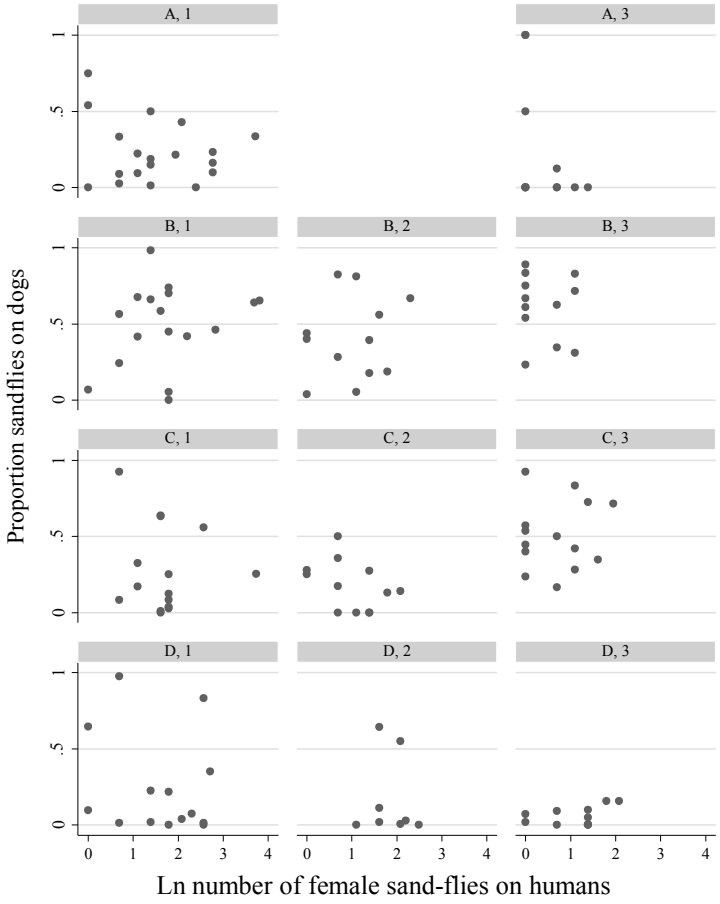


Figure 6.1: Relationship between female sandfly density on humans and the proportion of female sandflies on dogs in each house (I-L) during each trapping round (1-3).

GLMM analysis shows that the log-transformed household vector density has a significant positive linear association with FOI on dogs ($\beta=0.23$, $z=2.86$, $P=0.004$) and humans ($\beta=0.30$, $z=2.56$, $P=0.011$), indicating that FOI on both hosts increases with vector density, when the aforementioned fixed effect of trapping round and random effect of house ID are included within the model.

When effective chicken density is the sole predictor of FOI (controlling for trapping round and house ID), there is a significant nonlinear relationship between effective chicken density and FOI on dogs and humans. Here, the FOI on dogs decreases with

increasing effective chicken density (*β=-0.03, z=-3.36, P=0.001*), but latterly increases (*β=0.0003, z=2.10, P=0.036*). Similarly, an initial decrease in the FOI on humans is also associated with increasing effective chicken number (*β=-0.05, z=-3.23, P=0.001*) and is also followed by a marginal increase at higher effective chicken densities (*β=0.0004, z=1.78, P=0.075*), but this quadratic term only reaches borderline significance. Such nonlinearity is indicative of a change in shape of the relationship over host density. The quadratic predictors for both human (*β=0.0003, z=1.50, P=NS*) and canine FOI (*β=0.0002, z=1.65, P=0.098*), however, become non-significant once vector density is included within the model, thus quadratic predictors are excluded from subsequent analyses.

Analysis of the full FOI models, containing host density, vector density and round as fixed effects and house ID as a random effect, demonstrates a significant association between vector density and host density with the FOI on dogs and on humans. These analyses also reveal significant interaction between effective chicken density and vector density predictors in explaining the FOI outcomes (Table 6.5).

On humans the interaction effect is only borderline significant, therefore the main effects of host and vector density appear to be the most important predictors of FOI on humans.

Table 6.5: GLMMs to describe the association between effective chicken density and vector density with *ln* FOI on humans and dogs, taking into account fixed seasonal effects and structuring within the data.

Fixed effects	Category	Ln FOI on humans Co-efficient (95% CI)		Ln FOI on dogs Co-efficient (95% CI)	
Effective chicken density /ECD	0-15				
	16-30	-0.42	(-1.07, 0.24)	-0.33	(-0.72, 0.07)
	31-45	-0.81**	(-1.56, -0.05)	-0.42**	(-0.80, -0.03)
	46-60	-0.65	(-1.43, 0.14)	-0.45*	(-0.91, 0.003)
	>60	-0.84	(-1.85, 0.16)	-0.69**	(-1.26, -0.11)
Centred ln total vector density/LnTotal		0.36**	(0.11, 0.62)	0.30**	(0.13, 0.48)
Interaction	LnTotal X ECD0-15				
	LnTotal X ECD15-30	-0.15	(-0.63, 0.32)	-0.10	(-0.40, 0.20)
	LnTotal X ECD30-45	-0.26	(-0.085, 0.33)	-0.16	(-0.45, 0.14)
	LnTotal X ECD45-60	-0.58*	(-1.25, 0.09)	-0.65**	(-1.06, -0.25)
	LnTotal X ECD>60	-0.18	(-1.30, 0.94)	-0.16	(-0.73, 0.15)
Round	1				
	2	-0.23	(-0.89, 0.43)	-0.30	(-0.76, 0.15)
	3	-0.78	(-1.48, -0.08)	-0.06	(-0.43, 0.30)
Intercept		-0.20	(-0.67, 0.27)	0.52**	(0.06, 0.99)
Random effects		Estimate (95% CI)		Estimate (95% CI)	
House ID		0.04	(-0.07-0.15)	0.14	(-0.08, 0.37)

Note: **indicates significant at the P≤0.05 level; *borderline significance at the P≤0.1 level.

The relationship between canine FOI on dogs, the sandfly density and the effective chicken density is represented graphically (Figure 6.2). Interaction between these two predictors highlights that the effect of variation in vector density differs depending upon the density of hosts available (Table 6.5). In particular FOI on dogs is at its highest when sandfly density is high but the avaliability of alternative hosts

is low. Conversly, FOI on dogs appears to be at its lowest when both vector and host densities are very low, or very high.

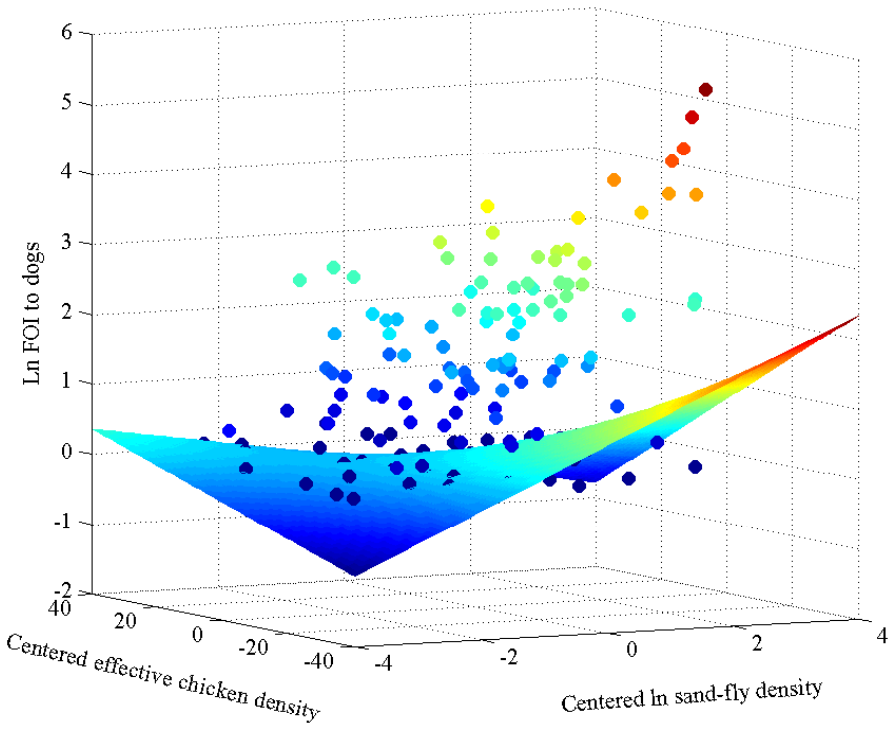


Figure 6.2: Surface plot of the average relationship between *ln* sandfly density, host (chicken) density and *ln* FOI on dogs calculated from regression coefficients, overlaid with data. Note that the model is corrected for household and round, but the data are not, so that there is not a correspondence in values. Heat map colour intensity of actual data points and simulated surface correspond to the *ln* FOI on dogs, with dark blue denoting low values and dark red high values. All host and vector density data are grand mean centred.

6.5. Discussion

This study represents one of the first attempts to elucidate the effect of chicken density upon leishmaniasis transmission. Overall, results suggest that there is a significant link between alternative host density and the absolute and relative preference of sandflies for humans and dogs. These investigations also indicate that

host choice has a vector density-dependent element, which varies significantly depending upon host density. Finally, host choice dynamics and vector density ultimately combine to indicate that under the assumptions made FOI on dogs and humans would also be influenced by host and vector densities, but that there are significant interactions between host and vector densities resulting in potentially complex FOI relationships.

Significant interaction between host and vector density in determining both the proportion of sandflies upon humans and dogs but also the FOI on dogs is indicative of a variable effect of host density at different vector densities. Critically, this results in high FOI on dogs when there are low chicken densities in combination with high vector densities, and a low FOI when both chicken density and vector density are jointly low or high.

These complex associations may well be explained by sandfly density-dependent aggregation behaviour as previously hypothesised in Chapter 6, whereby sandflies aggregate in response to sex pheromone production in association with hosts, which is led by host choice in response to host cues.

As vector density increases so does local pheromone production and levels of associated aggregation behaviour, however, sandflies require hosts on which to aggregate and form leks. In the absence of chickens, therefore, the options for aggregation locations are reduced, leading to increasing preference for readily available hosts, namely humans and dogs, as vector density increases. Yet, when chicken density is high it is likely that many flies will be attracted to chickens in

accordance with their biomass (Quinnell *et al.*, 1992), but that due to the synergistic effect of male sex pheromone production (Bray and Hamilton, 2007b) and the attraction of females to leks as a function of their size (Jones and Quinnell, 2002) this could result in increased lekking opportunities or "runaway preference" for chickens as vector density and pheromone production increases.

This may mean that the removal of chickens in areas of high vector density would cause the force of infection to significantly increase towards dogs and humans, a possibly undesirable consequence of chicken ownership policy in some Brazilian municipalities. These relationships may also mean that host density could be used to help divert sandflies from susceptible hosts at high vector densities, and act as a zooprophylactic agent. But, conversely, the addition of chickens in areas of low localised vector density may marginally promote transmission to dogs and humans, as there are only minimal aggregation dynamics in effect to encourage sandflies onto an alternative host. Yet, as data were sparse for low vector densities caution must be exercised in interpretation of the extremes of this interaction. The overriding observation therefore is that the local environment will be critical to the effect of chicken density manipulations and warrants further investigation.

Logically, transmission events from dogs to humans will not occur within a single night. However, given that host density was systematically varied between capture nights, it was necessary for the calculation of a variable proportional to the FOI on assume that the number and proportion of sandflies per host type on a given trapping night was indicative of the general trend. Given the mean trends in preference with vector and host density this is a justified and necessary assumption, but it is

important to be aware that high nightly variability in captures may influence outcomes.

Another implicit assumption of the FOI approximation presented here is that all flies have fed previously. In reality, the proportion of parous females (indicative of having taken a bloodmeal) may vary between one and two thirds of caught females over the season (Ferro *et al.*, 1995a). Infection rates also increase with sandfly parity, resulting in higher sandfly infection rates commonly observed at the latter end of the sandfly season (Ferro *et al.*, 1995a; Travi *et al.*, 1996). Therefore this highlights the need to investigate FOI separately in each round.

Host density dependence is clearly an important driver of host preference, and is a factor currently omitted from the analysis of data presented in Chapter 4, as it is subsumed into household random effects. However, using the relationship between host and vector preferences established here, future work could recalculate preference in the data from Chapter 4 households taking into account the consistent differences in chicken density, and thus provide an improved fit for the vector density-preference relationships in those data.

6.6. Conclusions

There appears to be important effects of and interactions between host and vector density in influencing absolute and proportional preference for dogs and humans. These effects have important implications for control and highlight the possibility of using chickens as a zooprophylactic agent. The benefits of chickens however, vary

significantly depending upon the local sandfly environment. Therefore, larger scale investigations including longer time series, sampling across wider ranges of sandfly density, and wider area (i.e. including neighbouring houses and other peridomestic sites), plus quantification of infection rate among sandflies are necessary to confirm the above dynamics and provide indication of under what conditions zooprophylaxis may be effective.

Chapter 7: **General discussion**

7.1. Introduction

The work presented in this thesis aimed to improve understanding of visceral leishmaniasis transmission and the opportunities for control, through the examination of existing prevalence data and field investigation of sandfly feeding preferences and their influencing factors.

Given that VL in the New World is caused by a zoonotic parasite (Desjeux, 2004), multiple host species are implicated in transmission to humans, and the domestic dog is considered the most important reservoir of infection within the peridomestic environment (Lainson and Rangel, 2005). A key assumption across zoonotic VL epidemiology is that transmission between dogs and humans, via the insect vector, is linearly related to one another (Dye, 1996). This assumption, if incorrect, would change the perceived interactions between all key vector and host components of the transmission cycle (not simply dogs and vectors), with potential far reaching impacts on current and future approaches to controlling transmission. Therefore this assumption and related issues were worthy of robust scrutiny.

This thesis focussed specifically on vector preference behaviour. Data on prevalence of exposure to *Leishmania* in co-localised human and dog populations were sought through examination of the literature and available data to identify patterns using statistical analytical and dynamic mathematical modelling methods. Experimental and observational data were then collected in the field in Brazil to test hypotheses of nonlinearity in sandfly biting preference. A discussion of study specific objectives,

findings, limitations and conclusions within the context of published work is presented at the end of each chapter. The aim in this Chapter is to summarise these findings in the broader context, and finally to make recommendations regarding future work.

7.2. Summary of findings

The relationship between human and canine prevalence of infection is not well understood, with a simple linear relationship between the two being conventionally assumed (Dye, 1996). One consequence of such an assumption is the view that halving the numbers of vectors results in halving of transmission rates to dogs and humans. This is clearly incorrect, and aggregations in parasite loads, host infectivity and vector distributions are well documented (Dye and Williams, 1995; Kelly, 2001; Kelly *et al.*, 1996; Woolhouse *et al.*, 1997). Such heterogeneities are rarely incorporated into statistical or population dynamic models of leishmaniasis or vector-borne diseases in general (Chaves and Hernandez, 2004; Chaves *et al.*, 2007; Dye, 1996; Reithinger *et al.*, 2004), apart from the innovative approach of Basáñez *et al.* (2007), expressly exploring for onchocerciasis, heterogeneities in *Simulium* preference for people as possible functions of vector and host density. Meta-analysis of a combination of published and unpublished zoonotic VL data indicates significant nonlinearity in the relationship between levels of exposure in dog and human populations as measured by immunological assays (Chapter 2). Humans can experience relatively low levels of exposure when canine prevalences are high, and statistically significantly lower than would be predicted by a linear positive relationship. In contrast, others suggest a linear relationship between human zoonotic

VL cases and canine seroprevalence (Assunçao *et al.*, 2001; Oliveira *et al.*, 2001), but these studies are based on human clinical case data which are known to give a very unreliable estimate of exposure to *L. infantum*. The ratio of exposed to symptomatic individuals varies widely between locations, due to a number of factors (Michalsky *et al.*, 2009), including immunological context (Alvar *et al.*, 2008), genetic backgrounds (Aagaard-Hansen *et al.*, 2010; Lipoldova and Demant, 2006), and socio-economic conditions (Alvar *et al.*, 2006; Dye and Williams, 1993). Surprisingly, little published data were available to characterise the human-canine exposure relationship, with few studies surveying both human and dog populations by comparable methods, and even fewer with concomitant entomological survey data. The current investigation represents the first attempt to compile a dataset for meta-analysis of host (past or present) infection relationships, and highlights the need for a more holistic approach to data collection in the study of zoonotic VL to permit further investigation of potentially complex host-prevalence relationships. In the future particular emphasis needs to be on reporting prevalence among vector populations too in order to fully qualify the relationships between host preference and prevalence in vertebrate hosts.

Investigation of the observed prevalence relationships using dynamics models (Chapter 2) demonstrates that the nonlinear and epidemiological patterns of infection in dogs and humans may be driven by nonlinearity vector biting rates, and therefore presumably infective biting rates, over vector density. In particular vector preference for dogs and humans appears to decrease at higher local vector densities, while simultaneously increasing on alternative hosts, like chickens. It was therefore hypothesised that the observed nonlinear pattern, relating dog to human prevalence,

could be explained, in part, by variation in biting preference. Experimental and observational field studies were undertaken to determine if feeding preferences for the three key peridomestic hosts (dogs, humans and chickens) varies with host and vector density. The ultimate aim is to incorporate these original data into a mathematical modelling framework. These field studies demonstrate only limited success in support of this hypothesis, however, due to difficulties in experimentally manipulating vector density and the high level of "noise" likely to affect any studies concerning vector densities.

In order to assess the distribution of sandflies between hosts, capture methods were optimised. Comparison of trap types (Chapter 3) revealed that there are significant differences in trap efficiencies, and that the use of a light source was a significant source of bias when catching flies in outdoor, unsheltered trap locations leading to the possible overrepresentation of outdoor hosts when comparing samples. Overall, these results demonstrate that CDCLT catch the most flies but require modification with a hood to reduce bias due to light when catching flies on dogs situated outside. The utility of CDCLT as a non-labour-intensive means of catching large numbers of sandflies is well known (Alexander, 2000), yet trap modifications on outdoor hosts to permit comparison of fly densities between locations while maintaining high capture rates represents a novel innovation in the capture of *Lu. longipalpis*. Further trap comparisons, of HLC and CDCLT inside houses (Chapter 3), demonstrate that sandfly capture is highly correlated by these two methods, and therefore CDCLT can be used for the estimation of human preference, similar to the associations between these two capture methods found among more endophilic vectors such as *P. papatasi* (Fryauff and Modi, 1991), *Lu. verrucarum* and *Lu. peruensis* (Davies *et al.*, 1995).

Experimental and observational field data (Chapter 4-6) demonstrates that flies were readily caught upon all hosts of interest, which confirms the eclectic nature of sandfly feeding preference in Marajó (Quinnell *et al.*, 1992). In general however, low numbers and proportions of flies were caught on humans; this is in contradiction to the high degree of anthropophily which has been occasionally reported for other *Lu. longipalpis* populations (de Oliveira *et al.*, 2008). Overall, the field data (Chapter 5-6) demonstrate the likely presence of vector density-dependent biting preferences on humans and dogs. The observed variation in sandfly densities was high, as has been reported previously (Kelly *et al.*, 1996; Quinnell and Dye, 1994a). Nevertheless, the general trends in distributions between hosts over a range of natural vector densities on both nightly and monthly scales demonstrate an important mean average effect in host preference in addition to the night-to-night heterogeneity. Namely, preference for reservoir hosts (dog), but in particular, "accidental" hosts (humans), decreases nonlinearly with increasing vector density, a relationship that is most marked when alternative host density is high (Figure 5.7). The generalizability of the results over temporal (seasonal) scales gives credence to the inclusion of such nonlinear relationships to improve mathematical models of zoonotic VL (see Chapter 2).

Changes in preference also appear to be driven by host density, as experimentally modified through the introduction and removal of chickens. Data indicate that the preference for dogs and humans decreased nonlinearly with chicken density, although nightly variation remained high. Previous comparisons of biting preference across relevant hosts (humans, dogs and chickens) have been carried out only

occasionally in the case of *Lu. longipalpis* (Quinnell *et al.*, 1992). The investigations of Quinnell *et al.* (1992) were not designed to detect complex effects, with only four nights of field data and lab estimation of preference carried out in the absence of male flies. Longer term investigations of sandfly preference, by others, have suggested possible switching in host preference over the seasons, though the causative mechanisms were not investigated (Morrison *et al.*, 1995a).

The pheromone mediated aggregation behaviour of sandflies is a biologivally plausible explanation for the nonlinearities observed in the field (Bray *et al.*, 2009; Bray and Hamilton, 2007b; Morton and Ward, 1989). In particular, the male produced sex pheromone attracts both females and male sandflies, which, due to the additional pheromone production from recruited males has the potential to act as positive feedback mechanism (Kelly and Dye, 1997), leading to highly aggregated and density dependant preference dynamics (Kelly and Dye, 1997; Ximenes *et al.*, 1999). This effect is the possible mechanism underlying density-dependent preference, although density dependent host defensiveness may also influence vector biting preference (Kelly and Thompson, 2000).

Density-dependent host switching has been identified in other vector species or groups, such as triatomine bugs, whereby feeding upon humans decreases with increasing dogs and vector density (Gurtler *et al.*, 2009; Gurtler *et al.*, 1997).

Where vectors are truly generalists, a bite on infection refractory species represents a failed transmission attempt by the parasite; therefore, according to a key assumption of the Ross- MacDonald model, the reproduction number (R_0) of a vector-borne

disease is influenced by the dilution of biting pressure upon competent hosts with increasing non-competent host densities. Thus, the addition of non-competent hosts will dilute the rate of contacts between vectors and susceptible hosts, thus resulting in nonlinear changes in the value of R_0, as the addition of each non-competent host has less of an (cumulative) effect upon the competent to non-competent host ratio (Woolhouse *et al.*, 2001). Modelling of cutaneous leishmaniasis led others to hypothesise possible nonlinearities in R_0 associated with the density of non-competent species (Chaves *et al.*, 2007). The nonlinearity observed over changing host densities described in this thesis lends credence to this suggestion in the case of zoonotic VL transmission. *Lu. longipalpis* sandflies do not, however, distribute themselves homogeneously between available hosts (Kelly *et al.*, 1996), therefore heterogeneity in host preference due to significant sandfly aggregation dynamics and vector density dependence should also be considered as key drivers (Kelly and Dye, 1997; Kelly *et al.*, 1996). Dilution is likely to play a part in transmission dynamics, but is too simplistic to explain the density-dependent preference observed among aggregating sandfly populations. An alternative, but non-mutually exclusive, interpretation of the observed nonlinear vector density dependence could be related to sandflies distributing themselves according to the ideal free distribution (IFD).

The ideal free distribution (IFD) of insect vectors, adapted from foraging theory (Sutherland, 1983), is based on density-dependent feeding success as the driving force behind the distribution of vectors. Specifically, vectors distribute themselves between hosts to achieve equal gains, determined by the trade-off between host quality and host defensive behaviour (Kelly and Thompson, 2000). Defensive behaviour is expected to be vector density dependent, as has been demonstrated by

linear reductions in feeding success in *Lu. longipalpis* (Kelly *et al.*, 1996), but is also expected to differ between hosts, as has been reported in *Culex* species (Fujito *et al.*, 1971; Kelly and Thompson, 2000). These features have the potential to drive nonlinear changes in biting preference with vector density as the "ideal" host, with respect to sandflies maximising gains of feeding success (to promote fecundity) and minimising interference from host defensive behaviour, may change with vector or host density. Estimating defensiveness parameters is difficult. The proportion of fully fed flies (fed to repletion) among all blood-fed flies has been used as an indicator in the past (Kelly *et al.*, 1996), but there is no standard measure of defensive behaviour, plus it was not within the scope of this thesis to address this underlying aspect of individual host choice.

If sandflies followed the IFD, the results presented here could potentially indicate low level defensive behaviour among humans and dogs at low vector densities, but strong increases in their defensiveness as vector density rises, while the defensiveness of chickens increases less rapidly, leading to a switch in sandfly preference.

Lu. longipalpis vectors of zoonotic VL do not appear to follow the IFD: Kelly *et al.* (1996) demonstrated that the defensive behaviour of chickens increases with biting pressure to result in an 80% reduction in the proportion of blood fed females which have fed to repletion as sandfly densities reached their peak. Yet this did not result in sandflies distributing themselves between individual chickens in a shed, or between sheds, to maximise resource gains and limit any differences in defensive behaviour which was not measured (Kelly *et al.*, 1996). Heterogeneity in individual

attractiveness may also contribute to aggregation on hosts of the same species (Kelly *et al.*, 1996), but aggregated distribution is also characteristic of the pheromone communication and co-localisation for mating activity in sandflies (Dye *et al.*, 1991; Kelly *et al.*, 1996). It is highly likely that female sandflies are simultaneously maximising their mating and feeding outcomes, so that the expected IFD is more complicated than when considering host defensiveness alone. Additionally, temporal differences in the commencement of male and female sandfly nightly activity, whereby males commence lekking before females (Kelly and Dye, 1997; Ximenes *et al.*, 2006), implies that defensiveness in response to biting activity is unlikely to impact the initial distribution of the first leks, which are set up subsequent to female fly recruitment and therefore contain only non-biting male sandflies.

Finally, IFD and host-specific gains are dependent on the assumption that flies are equal competitors, that the relative quality of each host is known and that there are no significant costs in moving between hosts (Crawley, 1992). Kelly *et al.* (1996) demonstrate no segregation of sandflies by competitive abilities, assuming size of females is an indication of competitive ability. Pheromone communication also implies assessment of host quality in terms of biomass and associated sandfly density via pheromone plumes before direct encounters enabling flies to make informed choices about host quality. However, this assumes that there are no directional or environmental factors effecting kairomone diffusion, which is unlikely to be correct. For example, wind speed was a significant factor for vector density in Chapter 3. Loyalty to hosts or locations where flies have experienced successful feeding is an alternative way to maximise feeding gains in a heterogeneous environment, and there is some evidence for site fidelity among New World sandfly

species, such as *Lu. whitmani* (Campbell-Lendrum *et al.*, 1999a), and Old World vectors such as *P. ariasi* (Killick-Kendrick and Rioux, 2002).

Overall, it may be that the aggregation-related costs to feeding success are at least modified by the benefits of lekking, which may reflect energetic gains in terms of co-localising mating and feeding activity (Yuval, 1994, 2006) and possible lek-related benefits of mate choice (Jones *et al.*, 2000; Jones *et al.*, 1998). Energetic gains are demonstrated by increased feeding behaviour among females within leks (Jarvis and Routledge, 1992), and increased propensity to mate once fully engorged (Chaniotis, 1967; Maroli, 1983). Benefits of mate choice are that attractive males breed attractive sons, leading to long term gains in female reproductive success (Jones *et al.*, 1998). Also middle-aged males are associated with greater offspring hatching success (Jones *et al.*, 2000). However, the relative trade-off between reproductive benefits of mate choice and host defensiveness and its possible role in sandfly distribution has not been exclusively tested, but represents an avenue for future work.

This indicates that the attractiveness of hosts, i.e. host quality in IFD terminology, is linked to male vector density and distribution in addition to host availability. This multifaceted relationship could potentially be incorporated within a calculation of the IFD by making both host quality *and* host defensiveness alter with vector density, although this could lead to a circular relationship without careful parameterisation. It was not, however, within the scope or timescale of this project to separate these effects by experiments in the field, but is proposed as a future research question.

Other sources of heterogeneity are also highly relevant to transmission; in particular heterogeneity in preference for individuals within a species (Hasibeder and Dye, 1988), especially if this refers to infectious dogs. Woolhouse *et al.* (1997) tested the statistical tenant that 20% of individuals are responsible for 80% of the transmission; an observation which has been qualified in numerous settings, such as in the preferential biting of mosquitoes on one of six human volunteers (Knols *et al.*, 1995), or the differential infectiousness of dogs naturally infected with *Leishmania* (Lanotte *et al.*, 1979). Xenodiagnosis studies by Courtenay *et al.* (2002b) demonstrated that 17% of the naturally infected cohort dogs in the Marajó villages of the current study were responsible for ≥80% of all transmission events. Heterogeneity of this kind has big implications for the improvement of effectiveness and efficiency of controls if such transmission "hot spots" could be readily identified, but similarly poor connotations for control if, through incomplete control, "hot spots" are missed. Preferential biting upon infected animals could also lead to nonlinearity in transmission, as could changes in biting rate among infected vectors as has been observed in both malarial (Ferguson and Read, 2004) and *Leishmania* vectors (Rogers and Bates, 2007), where infected flies bite more frequently.

It is possible that heterogeneity in bloodmeal size may promote such repeated feeding attempts, altering vector-biting rates. Reduction in bloodmeal size could potentially result from density-dependent feeding success (Kelly *et al.*, 1996), but also due to sandfly infection, whereby parasites block sandfly mouthparts preventing feeding to repletion (Rogers and Bates, 2007).

There is a range of other possible sources of nonlinearity in vector-borne disease transmission. For example, if all available vectors become infected then the transmission rate would differentially saturate, leading to nonlinearities in transmission. However, this is unlikely to occur in short-lived dipterans such as sandflies (Dye and Williams, 1995), where infection prevalence in sandflies populations is typically <1% (Sherlock, 1996). Significant infection-related vector mortality could also feedback to reduce vector population size via associated reductions in lifetime fecundity. Irrespective of the interaction between vector density and transmission rates, such processes are likely to lead to complex and possible cyclical dynamics in infection as vector lifespans are considerably shorter than those of the definitive host. Infection-related mortality is difficult to demonstrate among disease vectors in the field (Dye and Williams, 1995), where environmental factors may be the greater population limiting factor. Alternatively, vector mortality rate may be linked to parasite load, as has been observed in *Culex* mosquitoes in the transmission of *Wuchereria bancrofti* (Samarawickrema and Laurence, 1978), and *Simulium* spp. in onchocerciasis transmission (Basáñez *et al.*, 1996).

Within the host, interaction between exposure and immunity could also potentially produce nonlinear transmission dynamics, as has been seen in the dynamics of helminth infections (Schweitzer and Anderson, 1992).

Seasonality certainly influences vector density, and thus potentially host choice as described in this thesis (Chapter 4). Climate-induced sandfly survival may also influence the chance of a female sandfly taking multiple bloodmeals, thus influencing the chances of transmission. In European vectors ambient air

temperature has been shown to influence the extrinsic incubation period of *Leishmania* parasites (Rioux *et al.*, 1985), therefore at times of higher ambient air temperature *Leishmania* parasites may reach the infective metacyclic stage more rapidly, which combined with temperature dependent feeding rates and survival among some sandfly species, such as *P. perfeliewi transcaucasicus* in Iran, may lead to increased seasonal transmission (Oshaghi *et al.*, 2009). These too have consequences for infective sandfly geographical distributions (Maroli *et al.*, 2008).

7.3. Implications for control

Nonlinear density-dependent host preference relationships with respect to vector and host density undermine the simple assumptions of previous zoonotic VL disease models. In a seminal paper, Dye (1996) described *Leishmania* transmission in a multi-host system assuming a simple proportional relationship between infection in different host species, an assumption which has since been carried through *Leishmania* predictive modelling (Palatnik-de-Sousa *et al.*, 2001; Reithinger *et al.*, 2004). Transmission models are highly sensitive to vector-based parameters of mortality and host biting rate, as they appear multiple times within R_0 calculations. Host preference is a key component of effective biting rates, therefore nonlinearities and density dependence in this parameter will lead to very different estimates of infection in model outcomes. For example, the impact of insecticidal interventions is routinely included in models of control as an increase in sandfly mortality rate (Dye, 1996). Data presented here however indicate that changes in vector population size, associated with mortality, will have multiple effects; reducing the size of the biting population *and* altering the preference for infection-competent and non-competent

hosts. As such, density dependent preference may lead to an increased preference for dogs and humans as vector abundance is reduced by insecticide, which may be counterproductive in terms of control. Therefore, the possibility of variation, particularly nonlinear variation in host choice needs to be incorporated into future models of zoonotic VL.

However, the failure of intense "trapping out" in reducing vector density highlights that high sandfly emergence rates and/or immigration rates may limit the success of local vector density manipulations in a dynamic environment (Chapter 4). Rascalou *et al.* (2012) indicate that vector populations and transmission can be sustained by immigrating sandflies if local conditions are unsuitable for vector population maintenance. This ability of vector populations to withstand reductions in local adult abundance may undermine the effectiveness of controls such as insecticide applications, and may have contributed to the low success of spraying insecticide in chicken sheds to control sandflies on Marajó (Kelly *et al.*, 1997).

Another possible implication of density-dependent effects is that chickens could be used to manipulate vector preferences, drawing flies away from infectious and susceptible hosts (zooprophylaxis). In São Paulo (SP) state, the local health authorities' discourage the keeping of chickens to reduce noise and pollution spill-over into neighbours (Orin Courtenay, pers. comm.). This is predicted by others to have the added benefit of reducing vector density, both generally through reduction in sandfly feeding opportunities, and focally through reduced attractiveness (Alexander *et al.*, 2002). Yet, the results of this thesis suggest that in specific circumstances the removal of chickens could result in the loss of a sink blood source,

and potentially an important diversionary host, pulling sandflies away from infectious and susceptible hosts. Therefore chicken removal may be counterproductive in terms of controlling leishmaniasis transmission in some cases. Surprisingly, there are few studies to assess the effectiveness of zooprophylaxis.

Alexander *et al.* (2002) suggested that the attractiveness of chickens could recruit flies over large distances increasing household transmission (zoopotentiation), but also potentially putting humans and dogs at risk of biting and infection while flies are *en route*. Here, findings suggest that increasing chicken numbers do not necessarily impact the total number of flies per household; this may indicate that host manipulations have only a localised effect and that there is no significant change in recruitment to households. Without further data from surrounding households, however, it is not possible to comment on the effects of long-distance recruitment or diversion while flies are *en route*.

It is important to remain aware that these outcomes are dependent on the interaction between the absolute numbers of flies and host preference. If numbers of flies are not altered by host density (as appears to be the case in our experiments), then adding chickens is always likely to be beneficial. If, on the other hand, the numbers of flies is dependent on the number of hosts, then adding chickens can be beneficial or detrimental, depending on local conditions and the shapes of both biting preference and vector-host density relationships.

Finally, the importance of pheromone production in sandfly distribution points to the possibility that synthetic pheromone, as developed by (Hamilton et al. 1999a,

1999b) , would be a highly effective means of sandfly control (Hamilton, 2008). Pheromone release could mimic larger vector densities and encourage recruitment to the lure sites leading to increased mortality if there is insecticide present (Bray *et al.*, 2010). Yet according to vector density-dependent preference indicated in this thesis, the use of pheromone could potentially modulate preference and therefore transmission even when used without insecticide.

7.4. Study limitations

Taking a sample of local vector density inherently leads to the underestimation of vector abundance. Trapping across the three predominant peridomestic host species is likely to give a more reliable indication of abundance than any single location on its own (especially given the interactions between sites presented here) (Morrison *et al.*, 1993a). Nevertheless, there were still areas of the peridomestic environment which remained unmonitored. The relative contribution of sylvatic hosts to host preference, for example, remains unknown. Failure to appreciate local vector distribution can be pivotal, for example, failure of ITN to control anthroponotic VL in Nepal may be attributable to an incorrect assumption regarding the level of endophagy amongst the vector species, perpetuated by sampling sandflies at indoor locations only (Picado *et al.*, 2010b). Investigation of the distribution across all peridomestic hosts could have revealed the wider distribution of *P. argentipes*, as well as varying host preference. Preference for refractory hosts may also reveal an avenue for zooprophylaxis (Bern *et al.*, 2010).

An additional limitation of this fieldwork and a key assumption implicit to the preference data and calculation for a proxy of FOI is that the number of female flies caught upon a host is intrinsically related to the number of flies actually feeding on that host. Blood meal analysis from Morrison *et al.* (1993) confirms that a high percentage of flies caught on a host have indeed fed on that animal, with 91.8% of flies caught in pig pens reacting to porcine antiserum. This association looks to be most apparent in enclosed structures, where resting sites are in close proximity to the host of interest. The association may be less marked where there are exposed hosts, where flies may leave in search for appropriate resting sites, only to be caught at another location (Morrison *et al.*, 1993). This potential source of bias was addressed earlier in the protocol, by trap modification on the only abundant outdoor hosts, dogs (Chapter 3). Identification of blood meals in future experiments via molecular techniques (Burniston *et al.*, 2010; Sant'Anna *et al.*, 2008), would provide a better idea of the relationship between catch site and bloodmeal source.

Seasonality (Chapter 4) appears to be associated with important trends in vector density, but was divided up into trapping round in subsequent analyses (Chapter 6). Therefore, seasonal trends and possible interactions with host and vector density require attention in further investigations.

Effective chicken density (as used in Chapters 5 and 6) is largely determined by the diminishing function included within the calculation (see Chapter 5), therefore ECD varies considerably depending upon the values it is given. Further investigation and optimisation of this parameter is, therefore, required.

Host number per unit area (shed) was manipulated in Chapter 5. Sheds have previously been identified as an important predictor of vector density (Quinnell and Dye, 1994a), but critical questions remain over the contribution to preference dynamics made by the presence or absence of the animal shed itself. In the absence of a shed, animals host distributions may change within the peridomestic environment and become less aggregated, which on the basis of biomass may make isolated larger hosts, such as dogs and humans more attractive (Quinnell *et al.*, 1992). Differences in host aggregation could result in different sandfly dispersal patterns, and possible reduction of the zooprophylactic effects of chickens. The implications to host loyalty and dispersal therefore need to be investigated in the field.

Within the presented vector density data there are potential sources of bias resulting from the amalgamation of capture data from experiments 1 and 2 (Chapter 4). In particular, specific time periods (January) and households (A-D) are over represented within this dataset due to the differences in trapping regime. Despite the non-significant effect of trapping-out over this period, sustained trapping may still have resulted in some small reduction on vector density later on, potentially influencing analysis of seasonal variation. The relative scarcity of trapping nights with very high vector abundance over seasonal trapping may also lead bias within the results.

Finally, it is important to note that different *Lutzomyia* species and sibling species within vector complexes may exhibit differing feeding and aggregation behaviour; for example *P. argentipes*, the key vector of AVL in Southeast Asia also appears to lek in association with hosts (Lane *et al.*, 1990), whereas other New World sandflies

such as *Lu. umbratilis* do not (Balbino *et al.*, 2005) and others, such as *Lu. lenti* in Northeast Brazil, do not appear to communicate via pheromones (Hamilton *et al.*, 2002). Therefore, different species or sibling species may exhibit quite different density-dependent feeding preferences, meaning that the findings of this study are limited to *Lu. longipalpis*, vectors of zoonotic VL in the Northeast of Brazil.

7.5. Future work

Within the study household host density and blood meal availability do not appear to be a limiting factor on vector population density, as indicated by the relatively stable vector densities in each trapping round (Chapter 5). It is possible that vector population carrying capacity is determined by different factors in different locations, which may lead to alternative transmission dynamics if blood availability is limiting in terms of vector population size. Understanding differences in vector and host preference dynamics between locations is important to the application of interventions. Therefore, repetition of the above experiments in different ecological conditions could confirm reproducibility of these trends for regions of low and medium transmission intensity.

Additionally, experimental manipulation of vector density is critical to confirming the vector preference dynamics observed over the course of natural variation. Given the failure of trapping-out reported in this thesis, alternative or more intense interventions may be more effective in an area of lower baseline sandfly density, permitting the experimental estimation of the importance of vector density on feeding preference.

In explaining density dependence among sandflies, a novel model behind sandfly distributions is proposed, whereby both host quality *and* host defensiveness increase with vector density, and male sandflies significantly contribute towards the attractiveness of a host. Testing predictions of this mechanism via modelling and fieldwork approaches would represent a return to basic principles and permit better understanding of the driving mechanism behind the observed density-dependent effects, and would include the calculation of density-dependent defensiveness of different hosts, according to bloodmeal size (Kelly *et al.*, 1996).

The anti-feeding effects of insecticides may contribute to redistribution of vectors between hosts, without changes to vector density. Sheds and household structures are the typical target for insecticide residual spraying. There have been few studies to quantify potential repellency due to insecticide-treated materials e.g. sheds , ITN, dog collars, and consequent diversion to other hosts (Kelly *et al.*, 1997; Soremekun, 2008). Given density-dependent effects, repellency may be a critical component in determining the effect of certain interventions aimed at sandflies, therefore insecticide-induced behaviour in terms of diversion but also repellency and excito repellency warrant further investigation.

Finally, a key aim of future work is also to develop a model of zoonotic VL transmission incorporating multiple hosts and the density-dependent dynamics of sandfly preference behaviour, to develop a new framework for the examination of control options. This would build upon the intervention model put forward by Dye (1996) and permit the investigation of human and canine infection rates under

different integrated control conditions, whilst taking into account significant nonlinearities associated with host and vector densities, which may in themselves be intervention driven.

In addition to enhancement of the basic transmission model, developments should also be focussed upon improving the way in which interventions are simulated, for example taking into account the waning intervention effects, intervention intervals and coverage. In light of density-dependent host preference effects of particular interest should be host densities and the numerous effects of insecticide on vectors. Vector survival, vector population size and anti-feeding effects in the case of dog collars (Reithinger *et al.*, 2004) and ITN (Courtenay *et al.*, 2007), should all be exclusively modelled. Many insecticidal applications are also host specific, therefore interventions should be simulated with host-specific effects. Modelling the impact of pheromone-baited insecticidal traps represents a significant point of interest as pheromone traps will mimic vector density with potentially important implications for density-dependent host preference relationships and integrated control. However, the current lack of data on pheromone efficacy and longevity would make parameterising this particular intervention a significant challenge.

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Appendices

Appendix A: Summary of the causal agents of the leishmaniases, the associated vectors and their distribution (summarised from WHO (2010a)).

Clinical form	*Leishmania* species	Known/suspected vectors	Distribution	Known/suspected reservoir
VL, PKDL, CL	*L. (L.) donovani*	*P. argentipies, P. longiductus, P. orientalis, P. martini, P. celiae, P. alexandri, P. vansomerenae*	Africa, Southern Asia.	Human
VL, CL	*L. (L.) infantum*	*Lu. longipalpis, Lu. cruzi, P. wui, Lu. almerio, Lu. evansi, Lu. salesi, P. neglectus, P. perfiliewi, P. ariasi, P. tobbi, P. longicuspis, P. pernicious, P. kandelakii, P. balcanicus, P. transcaucausicus, P. chinensis, P. alexandri, P. langeroni, P. duboscqui, P. harpensis, P. syriacus, P. smirnovi, P. longiductus, P. pemiciosus, P. langeroni, P. halepensis,*	Central and South America, Southern Europe, areas of Western and Northern Asia and Western and Northern Africa.	Domestic dog, plus other species of fox, cat and opossum of the genus *Vulpes, Nyctereutes, Lycalopex, Cedocyon,* and *Didelphis*.
CL	*L. (L.) tropica*	*P. sergenti, P. saeveus, P. arabicus, P. guggisbergi, P. chabaudi, P. rossi, P. grovei*	Africa, Middle East, Southern Asia.	Human, but also species of dog and hyrax of the genus *Procavia* in North Africa region.
CL	*L. (L.) major*	*P. papatasi,*	Africa,	Various mouse and

		P. duboscqi. *P. bergeroti,* *P. salehi, P. anseril,* *P. caucasicus,* *P. mongolensis,* *P. bergeroti.*	Middle East,Western Asia.	gerbil rodent species including the genus *Rhombomys* *Meriones,* Psammomys, Gibillus, Arvicanthis, Tatera, Nesokis, Aethomys, and Arvicanthis.
CL, MCL, DCL.	*L. (L.) mexicana*	*Lu. olmeca olmeca, Lu. olmeca bicolor,* *Lu. colombiana,* *Lu. ayacuchensis,* *Lu. anthophora,* *Lu. diabolica.*	Central America	Various species of opossum and rodent of the genus *Didelphis,* *Heteromys,* and *Nyctomys.*
DCL	*L. (L.) pifanoi*	*Lu. flaviscutellata*	Venezuela	Unknown
CL, DCL	*L. (L.) amazonensis*	*Lu. flaviscutellata,* *Lu. longipalpis.* *Lu. reducta.*	South America	Various squirrel and rat rodent species including genus *Proechimys,* *Oryzomys,* *Wiedomys,* and *Sciurus.*
CL, DCL, MCL	*L. (L.) aethiopica*	*P. longipies,* *P. pedifer,* *P. sergenti,* *P. aculeatus.*	East Africa	Various species of hyrax of the genus Procavia, Heterohyrax, Dendohyrax, and Circetomys.
CL, DCL	*L. (L.) venezuelensis*	*Lu. olmeca bicolor.*	Venezuela	Unknown
CL	*L. (L.) killicki*	*Unknown*	North Africa	Unknown
CL	*L. garnhami*	*Lu. youngi.*	Costa Rica, Venezuela	Unknown
CL, MCL	*L. (V.) braziliensis*	*Lu. whitmani,* *Lu. youngi,* *Lu. neivai,* *Lu. migonei,* *Lu. ovallesi,* *Lu. nuneztovari anglesi,*	Central and South America	Dog, opossum and various rodent species including the genus, Didelphis, Rattus Melanomys,

		Lu. carrerai, *Lu. llanosmartinsi,* *Lu. shawi, Lu. pescei,* *Lu. ayrozai,* *Lu. yacumensis,* *Lu. wellcomei,* *Lu. intermedia,* *Lu. complexa,* *Lu. edwardsi,* *Lu. spinicrassa,* *Lu. colombiana,* *Lu. pia, Lu. towsendi,* *Lu. ylephiletor,* *Lu. cruciate.* *Lu panamaensis,* *Lu. tejadai,* *Lu. trinidadensis,* *Lu. spinicrassa.*		Micoureus, Akodon, Bolomys, Nectomis, and Thrichomys.
CL, MCL	*L. (V.) peruviana*	*Lu. peruensis,* *Lu. verrucarum,* *Lu. ayacuchensis.*	Peru	Dog, opossum, rodents of the genus *Phyllotis* and *Akodon*.
CL	*L. (V.) guyanensis*	*Lu. shawi,* *Lu, umbratilis,* *Lu. anduzei,* *Lu. whitmani,* *Lu. longiflocosa.*	South America	Species of sloth, opossum, anteaters and rodent of genus *Phyllotis*, *Didelphis*, *Tamandua*, and *Proechimys*.
CL, MCL	*L. (V.) panamaensis*	*Lu. trapidoi,* *Lu. gomezi,* *Lu. panamansis,* *Lu. yuilli,* *Lu. ylephiletor,* *Lu. hartmanni,* *Lu. cruciate.* *Lu. sanguinaria.*	Central America	Dog, sloth, opossum, rodent and porcupine of the genus Choloepus, Bradypus Metachirus, Didelphis, Heteromys, and Coendou.
CL	*L. (V.) lainsoni*	*Lu. nuneztovari anglesi,* *Lu. ubiquitalis.*	South America	Large rodent, *Agouti paca*.
CL	*L. (V.) naiffi*	*Lu. squamiventris,* *Lu. paraensis,*	South America	Armadillo, Dasypus

		Lu. amozonensis, Lu. ayrozai.		novemcinctus.
CL	*L. (V.) shawi*	*Lu. whitmani.*	Brazil	Various species of primate, coatis and sloth of the genus *Cebus, Chiropotes, Nasua, Bradypus,* and *Choloepus*.
CL	*L. (V.) colombiensis*	*Lu. hartmanni, Lu. panamaensis, Lu. gomezi.*	Northern South America	Hoffman's two-toed sloth, *Choloepus hoffmani.*
CL	*L. (V.) lindenbergi*	*Unknown*	Brazil	Unknown

Appendix B: Description of diagnostic tests and data collected in the survey of human and canine *Leishmania* exposure in Marajó, 1994-2005.

Study site and communities

These studies were conducted on endemic rural communities in the municipality of Salvaterra, Marajó Island, Pará state, Brazil (48°31'W, 0°46'S). Study details are outlined below.

Leishmanin skin test (LST) of humans

Leishmanin skin testing (LST) was carried out in May-August 1995 (Quinnell, unpublished) and in August-November 2004 (Courtenay, unpublished) using comparable methods and antigens, though different antigen batches. Briefly, household residents over 2 years old were tested using 0.1 ml of commercially available *Leishmania* antigen, which was injected intradermally into the lower left forearm. Control saline was administered 20cm above the *Leishmania* antigen on the same arm. Readings were taken 48 to 72 hours later by trained personnel. Following standard interpretation procedures, dermal indurations with a diameter of 5mm or

more were considered LST positive, inferring previous exposure to *Leishmania*. Visible reactions to the control saline (14 people in 2004) were treated conservatively as an inconclusive test and the participants excluded from the study.

Serological test in dogs

All available dogs were tested annually for *Leishmania* exposure by IFAT in 863 houses in 23 villages between 1992-1994 as previously described (Courtenay *et al.*, 1994) and in 1614 houses in 18 villages in April-May 2005 by ELISA (Courtenay, unpublished). 207 dogs from 141 of the 272 recruited households (51.8% of households) were tested for *Leishmania* infection by IFAT.

ELISAs: Briefly, serum was tested against crude *Leishmania* antigen harvested from *L. infantum* promastigotes (IFLA/BR/M6445). Results were expressed as arbitrary units calculated from a reference serum titrated on each plate, following others (Quinnell *et al.*, 1997).

Animal host densities

A census of household animal hosts (chickens and dogs) was conducted in 180 of 190 houses in 15 continuous villages by house-to-house visits by trained personnel between July-August 1992 (Quinnell and Dye, 1994) and again in July-October 2006 from a random selection of previously enumerated households in 18 villages (17.6% (SD 5.3) of houses from each community) (Courtenay, unpublished).

Ethical clearance

Approval was obtained from national and local ethical committees as described in the above studies. For studies conducted after 2004, ethical clearance was granted by the national Brazilian ethical committee (Coordenacao Nacional de Etica em Pesquisa CONEP 10041, 25000.046588/2004-19), the local animal ethical committee at Instituto Evandro Chagas, Belém (Comite de Etica em Pesquisa com Animais, CEPAN 001/2003), and the UK ethical committee in accordance with

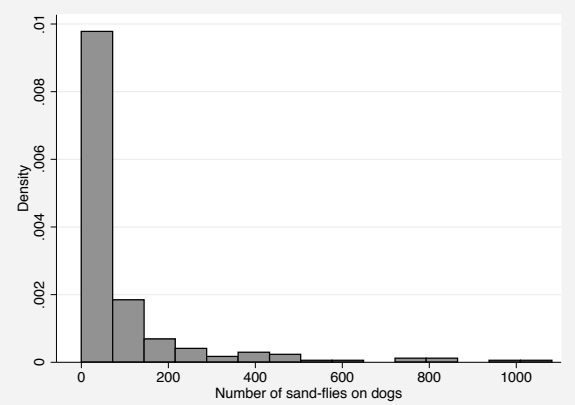
ICH/GCP guidelines (National Health Service [NHS] Research Ethics Committee, Warwickshire, RE557). Informed consent was obtained from humans and all dog owners prior to sampling.

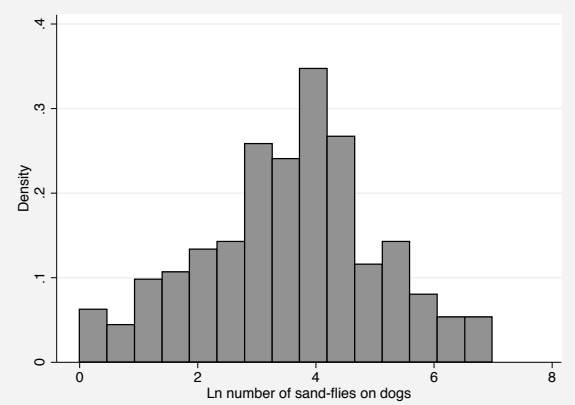
Appendix C: Age and sex adjusted human and canine prevalence across all study sites identified in Chapter 2, including geometric mean female sandfly data where available.

Data origin	No. of dogs tested	Adjusted No. positive dogs	Adjusted prevalence in dogs	95% CI	No. humans tested	Adjusted No. positive humans	Adjusted prevalence in humans	95% CI	Geometric mean no. of female sandflies per house	Geometric mean no. of female sandflies per shed
Courtenay (unpublished) 1	34	19.55	0.58	(0.34, 0.90)	96	55.17	0.57	(0.42, 0.75)	-	-
Courtenay (unpublished) 2	9	7.80	0.87	(0.34, 1.80)	86	28.55	0.33	(0.22, 0.48)	-	-
Courtenay (unpublished) 3	11	9.52	0.87	(0.41, 1.60)	52	35.11	0.68	(0.46, 0.94)	-	-
Courtenay (unpublished) 4	36	19.91	0.55	(0.34, 0.85)	209	90.90	0.43	(0.34, 0.54)	-	-
Courtenay (unpublished) 5	17	14.61	0.86	(0.49, 1.40)	114	32.08	0.28	(0.18, 0.40)	-	-
Courtenay (unpublished) 6	71	51.08	0.72	(0.53, 0.95)	639	284.32	0.44	(0.39, 0.49)	-	-
Courtenay (unpublished) 7	70	46.38	0.66	(0.49, 0.88)	481	185.45	0.39	(0.33, 0.44)	-	-
Courtenay (unpublished) 8	9	3.67	0.41	(0.08, 1.21)	115	38.40	0.33	(0.24, 0.45)	-	-
Courtenay (unpublished) 9	15	12.66	0.84	(0.45, 1.45)	91	33.64	0.37	(0.25, 0.51)	-	-
Courtenay (unpublished) 10	19	14.32	0.75	(0.40, 1.29)	170	81.89	0.48	(0.38, 0.59)	-	-
Courtenay (unpublished) 11	18	10.62	0.59	(0.28, 1.09)	163	40.96	0.25	(0.17, 0.34)	-	-
Courtenay (unpublished) 12	39	22.49	0.58	(0.36, 0.88)	232	85.07	0.37	(0.29, 0.45)	-	-
Courtenay (unpublished) 13	15	11.11	0.74	(0.38, 1.30)	120	54.63	0.46	(0.34, 0.59)	-	-
Courtenay (unpublished) 14	19	14.68	0.77	(0.43, 1.28)	120	51.39	0.43	(0.31, 0.56)	-	-
Courtenay (unpublished) 15	3	2.03	0.68	(0.06, 2.49)	98	42.06	0.43	(0.30, 0.58)	-	-
Courtenay (unpublished) 16	4	4.04	1.01	(0.26, 2.61)	76	13.08	0.17	(0.09, 0.29)	-	-
Courtenay (unpublished) 17	8	3.71	0.46	(0.12, 1.20)	55	23.44	0.43	(0.27, 0.62)	-	-
Courtenay (unpublished) 18	34	32.78	0.96	(0.67, 1.35)	268	123.52	0.46	(0.38, 0.55)	-	-
Quinnell (unpublished) 1	6	3.70	0.62	(0.16, 1.59)	21	13.98	0.67	(0.35, 1.14)	14.50	345.84
Quinnell (unpublished) 2	19	10.77	0.57	(0.28, 1.01)	108	65.51	0.61	(0.46, 0.77)	6.31	180.27
Quinnell (unpublished) 3	7	5.92	0.85	(0.33, 1.75)	40	15.43	0.39	(0.22, 0.61)	1.98	53.51
Quinnell (unpublished) 4	10	5.24	0.52	(0.18, 1.14)	33	5.50	0.17	(0.05, 0.36)	1.18	34.07
Quinnell (unpublished) 5	5	2.78	0.56	(0.10, 1.64)	26	8.78	0.34	(0.14, 0.66)	2.42	13.65
Quinnell (unpublished) 6	6	4.25	0.71	(0.22, 1.66)	27	3.03	0.11	(0.02, 0.33)	2.10	39.76
Quinnell (unpublished) 7	12	5.41	0.45	(0.16, 0.98)	50	10.24	0.20	(0.09, 0.39)	4.01	11.97
Quinnell (unpublished) 8	8	3.54	0.44	(0.11, 1.14)	19	9.88	0.52	(0.24, 0.96)	18.51	151.44
Quinnell (unpublished) 9	18	10.94	0.61	(0.28, 1.12)	81	37.19	0.46	(0.32, 0.63)	5.52	236.16
Quinnell (unpublished) 10	16	7.21	0.45	(0.19, 0.89)	32	10.68	0.33	(0.17, 0.58)	2.08	62.23
Evans *et al.* (1992) site 1	75	42.00	0.56	(0.44, 0.67)	193	75.38	0.39	(0.32, 0.46)	-	-
Evans *et al.* (1992) site 2	42	21.00	0.50	(0.34, 0.65)	193	90.60	0.47	(0.39, 0.54)	-	-
Evans *et al.* (1992) site 3	80	31.00	0.39	(0.28, 0.50)	193	27.22	0.14	(0.09, 0.19)	-	-
Cunha *et al.* (1995) site 1	15	7.00	0.46	(0.21, 0.73)	152	49.14	0.32	(0.24, 0.40)	-	-
Falqueto *et al.* (2009) site 1	109	62.00	0.57	(0.47, 0.66)	277	92.08	0.33	(0.27, 0.39)	-	-

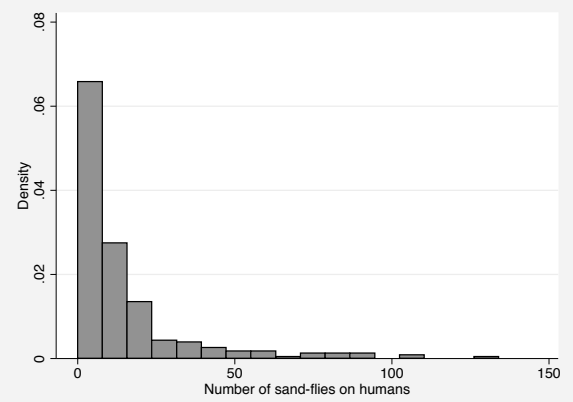
Appendix D: Frequency histograms of the untransformed and then log-transformed sandfly nightly count data on dogs (a), humans (b) and chickens (c), collected during the trap optimisation experiment (Chapter 3).

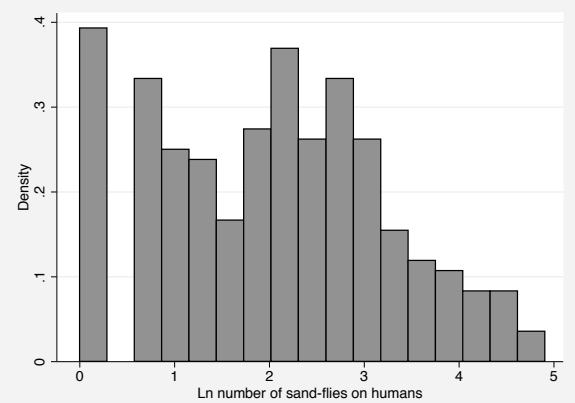
a



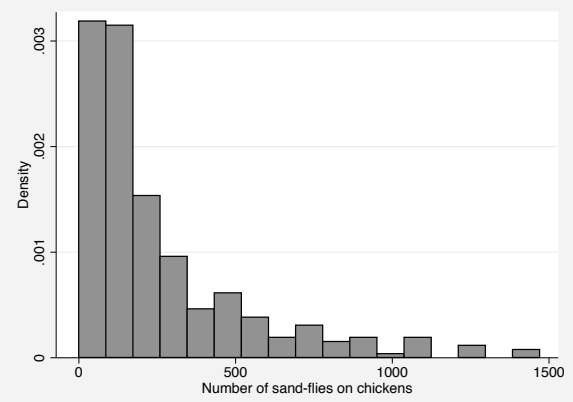


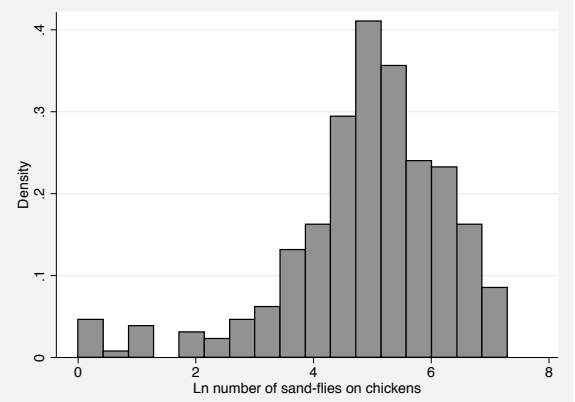
b





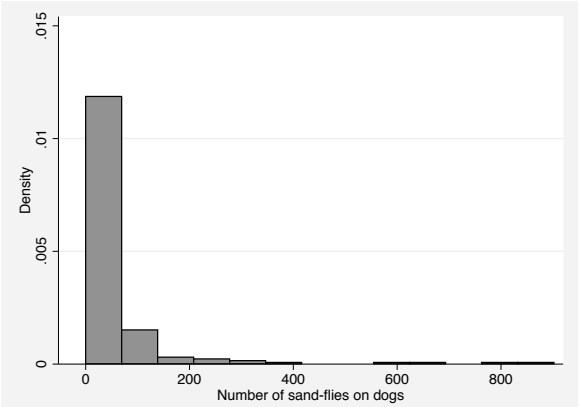
c

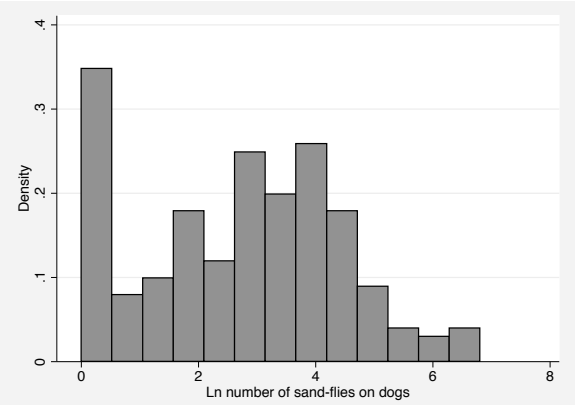




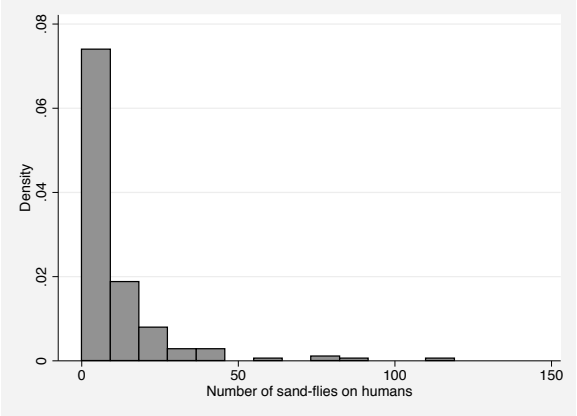
Appendix E: Frequency histograms of the untransformed and then log-transformed sandfly nightly count data on dogs (a), humans (b) and chickens (c), collected over the manipulation of chicken density (Chapter 5 and 6).

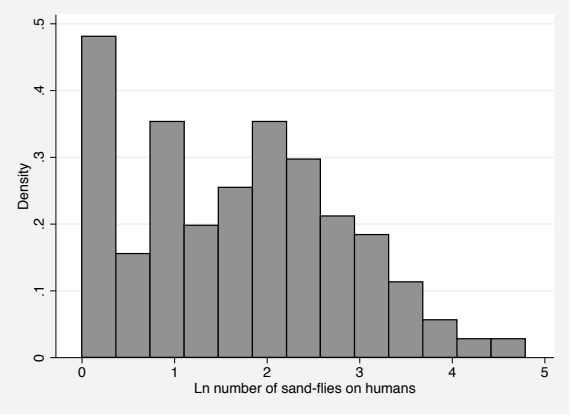
a



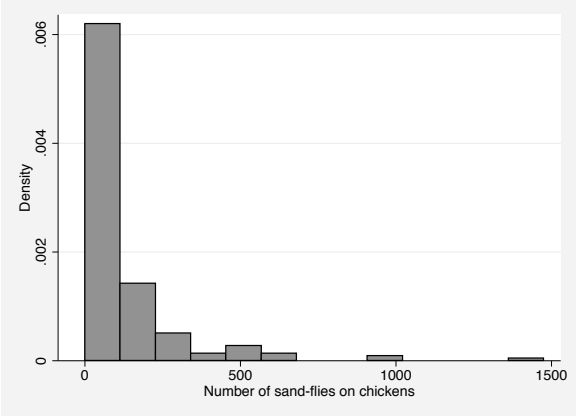


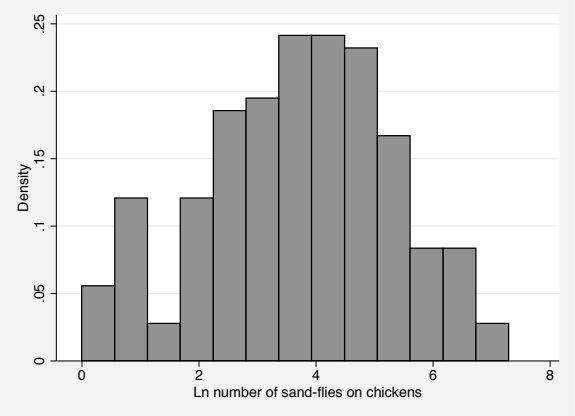
b





c





Appendix F: Correlation matrix of significant covariate predictors of sandfly density upon dogs, humans or chickens when comparing trap types (Chapter 3). Highly collinear variables ($\beta \geq$ +/- 0.8) are highlighted in grey.

	No. adults	*No. child-ren*	*No. adults at trap*	*No. human at trap*	*No. large chickens*	*No. ducks*	*No. dogs*	*No. dogs away*	*Roost height*	*Mean dist-ance*	*Vege-tation _2*	*Wall type _3*	*Bednet _1*	*Rain during day_1*	*Wind _1*	*Wind _2*	*Wind _3*	*DogID_2*	*DogID_3*	*DogID_4*	*DogID_5*	*DogID_6*	*DogID_7*
No. adults	1.0																						
No. children	0.4	1.0																					
No. adults at trap	0.2	-0.5	1.0																				
No. humans at trap	0.2	-0.4	1.0	1.0																			
No. large chickens	-0.3	0.6	-0.6	-0.6	1.0																		
No. ducks	-0.4	0.3	-0.3	-0.2	0.5	1.0																	
No. dogs	-0.1	0.3	0.0	0.0	0.2	0.8	1.0																
No. dogs away	-0.1	0.4	-0.1	0.0	0.2	0.8	1.0	1.0															
Roost height	0.1	0.8	-0.5	-0.4	0.7	0.6	0.6	0.6	1.0														
Mean distance	-0.3	0.5	-0.4	-0.3	0.7	0.9	0.8	0.9	0.8	1.0													
Vegetation_2	-0.3	0.4	-0.3	-0.2	0.5	0.9	0.9	0.9	0.6	1.0	1.0												
Walltype_3	0.3	-0.4	0.3	0.2	-0.5	-0.9	-0.9	-0.9	-0.6	-1.0	-1.0	1.0											
Bednet_1	0.3	-0.4	0.3	0.2	-0.5	-0.9	-0.9	-0.9	-0.6	-1.0	-1.0	1.0	1.0										
Rain during day_1	0.0	-0.1	0.0	0.0	-0.1	-0.1	0.0	-0.1	-0.1	-0.1	-0.1	0.1	0.1	1.0									
Wind _1	-0.1	0.0	-0.1	-0.1	0.0	0.0	0.1	0.1	0.0	0.1	0.1	-0.1	-0.1	-0.3	1.0								
Wind _2	0.1	0.2	0.0	-0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	-0.2	-0.3	1.0							
Wind _3	0.0	0.1	-0.2	-0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	-0.5	-0.3	1.0						
DogID_2	-0.3	0.4	-0.2	-0.1	0.5	0.9	0.9	0.9	0.6	0.9	1.0	-1.0	-1.0	0.0	0.0	0.0	0.1	1.0					
DogID_3	0.2	-0.1	0.2	0.2	-0.3	-0.1	0.0	0.0	-0.1	-0.2	-0.1	0.1	0.1	-0.1	0.0	0.1	0.0	-0.1	1.0				
DogID_4	0.3	-0.2	0.3	0.3	-0.6	-0.2	0.1	0.0	-0.2	-0.3	-0.2	0.2	0.2	0.1	0.2	0.0	-0.2	-0.2	-0.1	1.0			
DogID_5	0.3	-0.1	0.3	0.3	-0.4	-0.2	0.0	0.0	-0.1	-0.2	-0.2	0.2	0.2	0.0	-0.2	-0.1	0.0	-0.2	-0.1	-0.1	1.0		
DogID_6	0.2	0.5	-0.4	-0.4	0.5	-0.3	-0.5	-0.5	0.4	-0.2	-0.4	0.4	0.4	-0.1	-0.1	0.1	0.0	-0.3	-0.1	-0.2	-0.2	1.0	
DogID_7	-0.4	-0.7	0.2	0.2	-0.2	-0.3	-0.5	-0.5	-0.8	-0.4	-0.3	0.3	0.3	0.1	0.0	0.0	0.0	-0.3	-0.1	-0.2	-0.1	-0.3	1.0

Note: _n indicates use of a dummy variable as derived from categorical predictors