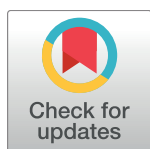REVIEW

Combining epidemiology with basic biology of sand flies, parasites, and hosts to inform leishmaniasis transmission dynamics and control

Orin Courtenay[1,2]*, Nathan C. Peters[3], Matthew E. Rogers[4], Caryn Bern[5]

1 School of Life Sciences, University of Warwick, Coventry, United Kingdom, **2** Zeeman Institute, University of Warwick, Coventry, United Kingdom, **3** Snyder Institute for Chronic Diseases, University of Calgary, Calgary, Alberta, Canada, **4** Department of Disease Control, London School of Hygiene and Tropical Medicine, London, United Kingdom, **5** Department of Epidemiology and Biostatistics, School of Medicine, University of California San Francisco, San Francisco, California, United States of America

* orin.courtenay@warwick.ac.uk

Abstract

Quantitation of the nonlinear heterogeneities in *Leishmania* parasites, sand fly vectors, and mammalian host relationships provides insights to better understand leishmanial transmission epidemiology towards improving its control. The parasite manipulates the sand fly via production of promastigote secretory gel (PSG), leading to the "blocked sand fly" phenotype, persistent feeding attempts, and feeding on multiple hosts. PSG is injected into the mammalian host with the parasite and promotes the establishment of infection. Animal models demonstrate that sand flies with the highest parasite loads and percent metacyclic promastigotes transmit more parasites with greater frequency, resulting in higher load infections that are more likely to be both symptomatic and efficient reservoirs. The existence of mammalian and sand fly "super-spreaders" provides a biological basis for the spatial and temporal clustering of clinical leishmanial disease. Sand fly blood-feeding behavior will determine the efficacies of indoor residual spraying, topical insecticides, and bed nets. Interventions need to have sufficient coverage to include transmission hot spots, especially in the absence of field tools to assess infectiousness. Interventions that reduce sand fly densities in the absence of elimination could have negative consequences, for example, by interfering with partial immunity conferred by exposure to sand fly saliva. A deeper understanding of both sand fly and host biology and behavior is essential to ensuring effectiveness of vector interventions.

Author summary

We review recent research that sheds light on the quantitative biology of leishmanial transmission between sand flies and mammalian hosts and use these insights to better understand transmission, the observed epidemiology of the disease, and their implications in choice of control strategy. Using animal models, we show how the parasite-induced

processes manipulate sand fly blood-feeding behavior and the infectious metacyclic dose to promote host infection and to differentially regulate the onward transmission potential of individual vectors and hosts. The existence of subpopulations of mammalian and sand fly "super-spreaders" provides a biological basis for the spatial and temporal clustering of clinical leishmanial disease. While tools are unavailable to distinguish these individuals in mixed populations, blanket interventions will be necessary to ensure inclusion of transmission hot spots. Interventions that reduce sand fly densities without elimination could interfere with vector—host dynamics and conferred partial immunity to host populations.
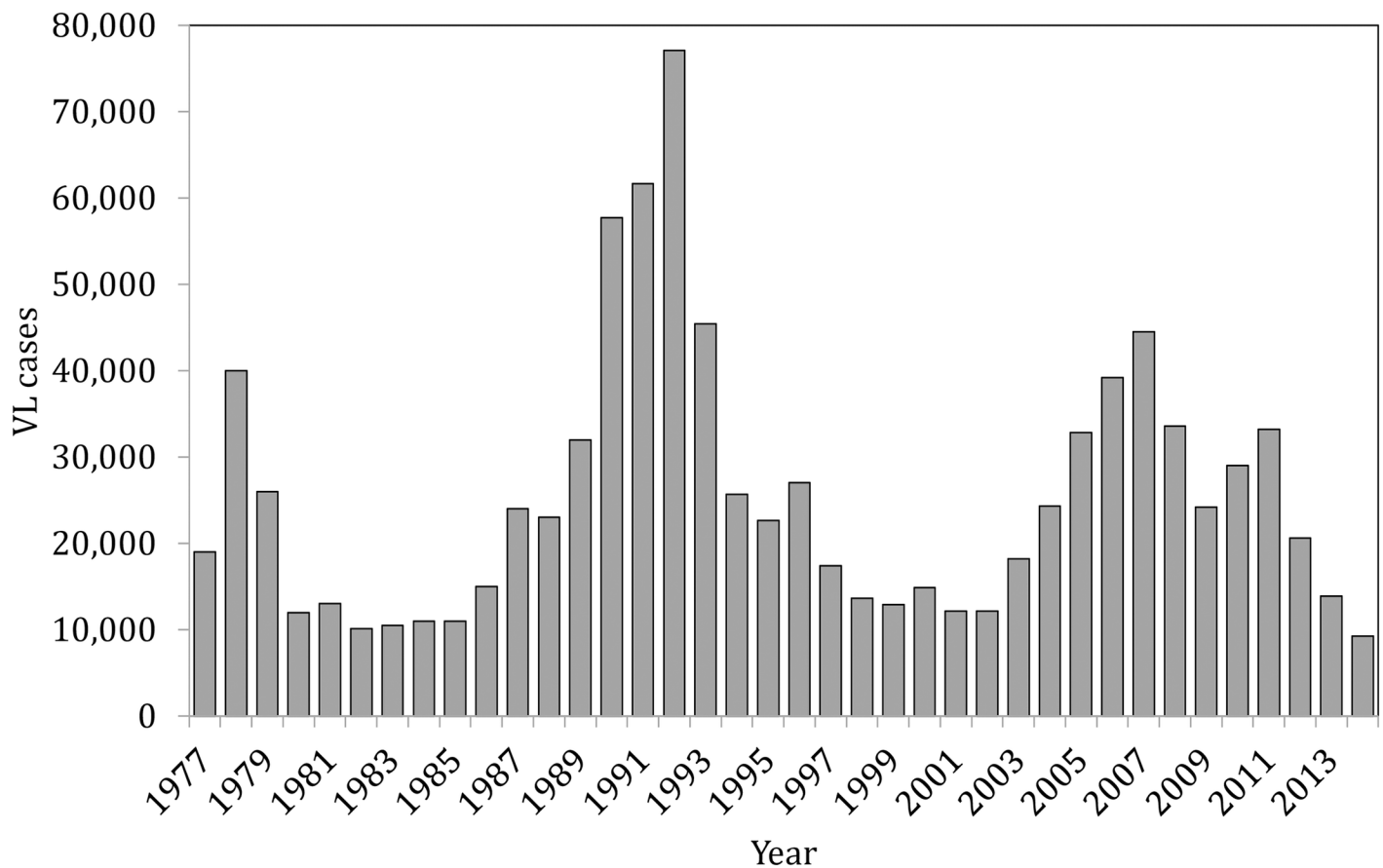
Introduction

In the Indian subcontinent, an effort to eliminate anthroponotic visceral leishmaniasis (VL) has been underway for a decade, and the incidence of the most severe clinical form, kala-azar, is at its lowest levels in 45 years. The program appears to be on track to "eliminate VL as a public health problem" by 2020 (defined as kala-azar incidence <1 case per 10,000 population) [1]. However, true elimination of transmission will be more elusive and requires a deeper understanding of the biology underlying transmission and disease. Substantial VL and cutaneous leishmaniasis (CL) burdens occur in many other continents, but the transmission dynamics and reservoir hosts differ, and development of tools for control and elimination are less advanced than in South Asia [2]. In this article, we review recent research that sheds light on the quantitative biology of leishmanial transmission between sand flies and mammalian hosts and use these insights to better understand observed patterns of VL and CL transmission and disease.

Leishmaniasis clusters in time and space

Since VL was first studied in India nearly a century ago, investigators have observed incidence cycles that rise and fall with a slow periodicity [3]. Cycles have been documented in India, Bangladesh, Sudan, and Brazil [4–8]. A single cycle tends to last 5 to 15 years, with interepidemic intervals of 10 to 30 years [4, 5, 8]. At a regional level, climatic factors may contribute to these periodic cycles [9]. In a community, the fall in incidence after several peak years is thought to result from the buildup of herd immunity, with new epidemic onset occurring when a sufficient number of susceptible residents have accumulated through births and/or in-migration [3, 6]. The current best measure of protective immunity is the leishmanin skin test (LST), which reflects durable cell-mediated immunity. Individuals with a positive LST have more than 95% lower risk of kala-azar compared to those with negative LST, and the age-related rise in positive LST prevalence parallels an age-related decrease in disease risk [10–12]. In contrast, exposure to infective sand flies is variably age dependent [12, 13]. A fall in the average age of kala-azar patients may be observed as an epidemic matures [14]. The level of herd immunity required to end an epidemic cycle and the time to reach this level likely vary depending on parasite virulence, transmission intensity, vector exposure patterns, and host factors such as nutritional status and access to treatment. Interventions such as vector control and rapid case detection and treatment may alter the cycle but have not been shown to eliminate the periodicity. Intensive blanket DDT spraying during the malaria eradication program of the 1950s–1960s prolonged the interepidemic period in the Indian subcontinent, but since the resurgence in the 1970s, there have been 3 typical epidemic cycles in India and 2 in Bangladesh (Fig 1) [6, 15–18].

Periodic epidemic cycles represent clustering in time; the second major characteristic of VL epidemiology is clustering in space. On a global scale, VL is highly clustered, with 90% of the

Fig 1. Reported cases of visceral leishmaniasis in India from 1977 to 2014. Data 1977–1985 are from Bihar only; data from 1986 onward include all reported cases in India. *Source of data: Ministry of Health and Family Welfare, Government of India, as published in* [17, 18].

https://doi.org/10.1371/journal.ppat.1006571.g001

disease burden occurring in relatively few states or districts within just 6 countries: India, Bangladesh, Sudan, South Sudan, Brazil, and Ethiopia [4]. At smaller spatial scales, VL-affected communities and census tracts cluster in space and time [19, 20]. At the most local scale, strong clustering is seen at the household and near-neighbor levels [20–23]. Small-scale clustering is most marked early in an epidemic cycle when most community residents are susceptible and tends to disappear as the prevalence of immunity rises [21]. Clustering is likely due to macro- and microenvironmental conditions that promote sand fly breeding, survival, and aggregation, including proximity to reservoir and nonreservoir blood sources (humans, dogs, or other animals) [9, 19, 20, 24]. Sand fly aggregations are mediated by complex host—sand fly interactions (e.g., [25]) including sex-aggregation pheromones released by males of some species [26, 27], host kairomones, or plant phytochemical attractants [28]. Temporal clustering of fly infection prevalence is often greatest in the wet season or at the end of the "sand fly season", when few nulliparous females are emerging and physiological sand fly age is greatest (measured by parity) [29]. Variation in the vector's propensity to blood feed indoors or outdoors may also determine who receives the most infectious bites.

Mammalian infection reservoirs

Household and near-neighbor clustering supports the assumption that untreated kala-azar cases, long known to be infectious to sand flies [30], comprise the most important infection

reservoir fueling transmission during epidemics. Post-kala-azar dermal leishmaniasis (PKDL), a chronic dermatosis that follows apparently successful kala-azar treatment in 5% to 15% of patients in the Indian subcontinent and up to 50% in Sudan, is thought to provide the reservoir that maintains transmission between epidemic cycles [6, 31–33]. PKDL patients are usually not systemically ill, may remain untreated for years, and have been shown to be infectious to sand flies [6, 33–36]. Demonstration of infectiousness requires feeding of laboratory-reared sand flies on the patient (direct xenodiagnosis) or the patient's blood via a membrane feeder (indirect xenodiagnosis). Because xenodiagnosis is impractical for population-based studies, investigators have sought proxy measures, such as quantitative polymerase chain reaction (qPCR), but the strength and shape of those relationships in different hosts require validation.

In canine leishmaniasis, positive serology or qPCR had high sensitivity (97%–100%) to identify highly infectious dogs but low specificity (13% for serology, 22% for qPCR in ear skin biopsy) [37]. A derived threshold cutoff in ear skin showed sensitivity of 100% to predict highly infectious dogs and specificity of 98% to identify noninfectious ones [37]. Although clinical VL severity was significantly associated with infectiousness, parasite load using the cut-off was a better predictor. These and other canine data also clearly demonstrate that some dogs are "super-spreaders" while others contribute little to transmission: in published xenodiagnosis studies, 15% to 44% of dogs were responsible for >80% of all sand fly infections [38–40]. No such proxies have been validated in human leishmaniasis, although preliminary data from 3 PKDL patients suggest that parasite loads in skin biopsies may provide a proxy for infectiousness [36].

Asymptomatic infection

Currently, a major question facing VL control efforts in the Indian subcontinent is whether persons with asymptomatic infection are sufficiently infectious to sand flies to constitute an epidemiologically significant infection reservoir [41]. Asymptomatic infections based on sero-conversion outnumber clinical disease by 4- to 17-fold, with rising ratios as kala-azar incidence falls [23, 32, 42–45]. If even a subset of asymptomatic individuals are infectious at a very low level, they could still play an important role in transmission, especially when clinical disease incidence is driven to low levels [46, 47]. Failure to address this potential reservoir could preclude interruption of transmission [1].

Recent Indian data show that the median blood parasite load by qPCR is 500-fold higher in kala-azar patients than in asymptomatically infected individuals [48]. Data from the same group confirm that parasite loads in peripheral blood correlate well with those in splenic aspirates [49]. High parasite loads were rare among asymptomatic infections and, when present, indicated individuals with high risk of subsequent development of kala-azar [50]. Antibody titers may also help distinguish asymptomatic infection from "presymptomatic" infection. In a longitudinal study in India, 12% of those with direct agglutination test (DAT) titers >25,600 subsequently developed clinical disease, compared to 1% of those with low titer positive DAT results [44].

In canine leishmaniasis, asymptomatic infected dogs are expected to be less infectious than polysymptomatic dogs through time [38, 51], whereas in naturally infected wildlife hosts, infection is usually benign and associated with relatively low parasite loads and degree of infectiousness (e.g., foxes in Brazil [52] and lagomorphs in Spain [24]). However, asymptomatic animals may have a longer infectious life expectancy than diseased, highly infectious individuals. The canine data reviewed above [37] suggest that qPCR has the most promise as a proxy for xenodiagnosis, but that relationship may vary with parasite tropism and *Leishmania* species [53]. The best specimen type (e.g., peripheral blood or skin biopsy), quantitative technique,

and threshold will need to be rigorously validated against xenodiagnosis as the gold standard in each epidemiological setting.
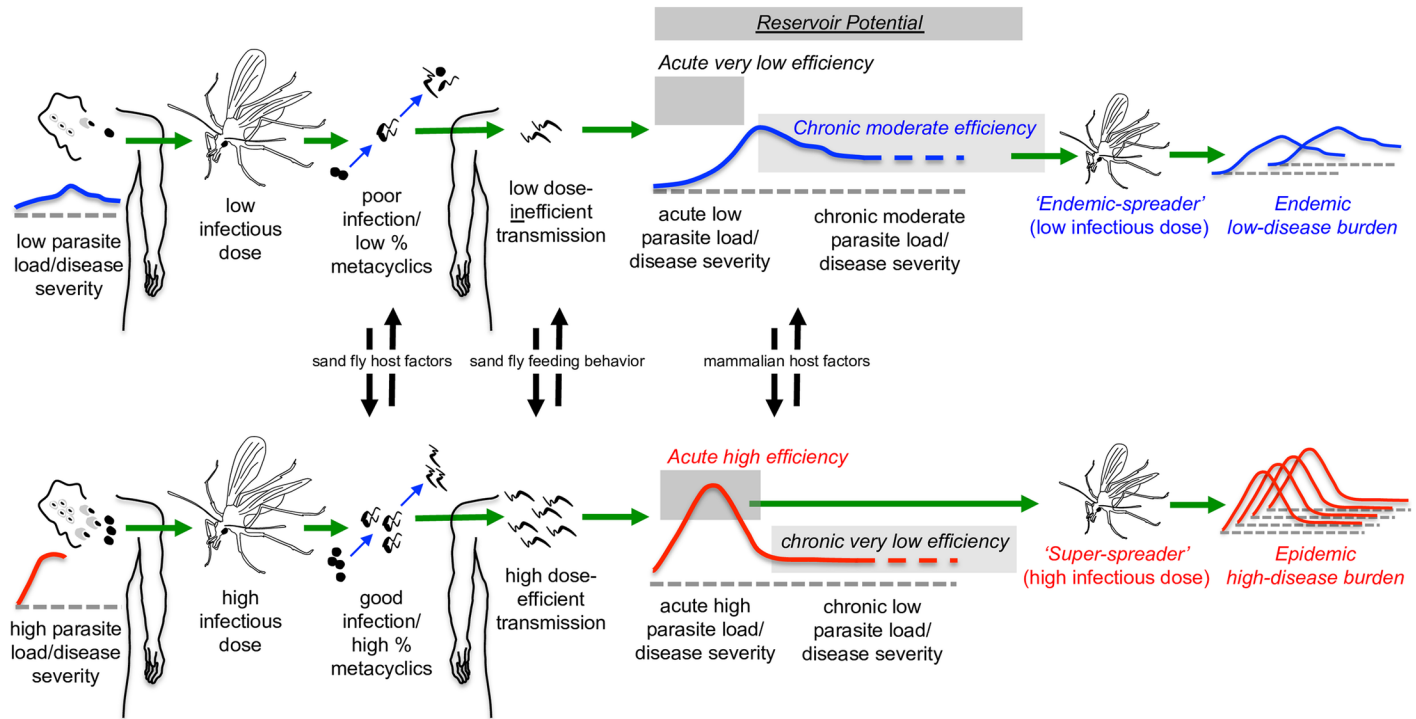
Influence of sand fly infecting dose on the efficiency of subsequent transmission

In nature, sand flies likely become infected with varying doses of parasites. This initiating dose [54], combined with sand fly immunity, parasite virulence, the sand fly gut microbiota [55–57], environmental conditions, and the blood meal [34], influences parasite development in the gut and subsequent transmission. In particular, the sand fly gut microbiota has recently been shown to heavily influence parasite survival [55–57] and transmission [56]. In an experimental model, transmission via flies infected with varying doses of *L. major* parasites was quantified [54]. Higher infecting inocula resulted in greater numbers of parasites per sand fly on day 14 postinfection and higher percentages of metacyclic promastigotes. The percentage of metacyclics was the best predictor of subsequent transmission efficiency to the mammalian host. The bites of high-dose infected flies resulted not only in higher transmission frequencies but also increased disease severity. Temperature, humidity, and oviposition status also significantly influenced transmission efficacy [54]. These observations support the concept, as described for dogs, of "super-spreader" blood meal hosts with high parasite loads resulting in flies with high-dose infections that initiate more severe infections upon subsequent transmission.

High versus low inocula have differing acute and chronic transmission characteristics

In an analysis of transmission by single sand flies, most infected mice were inoculated with a low dose (<600 parasites); however, for 1 in 4, the inoculum was >1,000 parasites. High-dose transmission resulted from heavy midgut infections, incomplete blood feeding, and transmission of a high percentage of the parasite load from the fly [41]. In a related analysis, low-volume (5-uL) injection of low (100) or high (5,000) doses of sand fly—derived metacyclic promastigotes were inoculated into a restricted dermal site in mice that had been preexposed to sand fly bites. Inoculation of 5,000 parasites into the ear dermis resulted in higher initial parasite loads and more severe acute disease. However, high-dose infections resolved more completely, with a lower lesion size during the chronic phase and a trend towards lower parasite numbers in the skin. Similar observations were published by Lira et. al [58]. Several studies have allowed uninfected flies to feed on the site of primary *L. major* infection. As expected, the parasite load in the dermal site of infection directly correlated with the efficiency of transmission from the mammalian host to the vector, with very low parasite loads typically failing to transmit [58–60]. Although the more severe lesions observed at early time points in mice receiving high dose inocula resulted in highly efficient transmission to uninfected flies, these lesions were less efficient at chronic time points. In contrast, lesions initiated with low doses did not result in transmission back to sand flies during early infection but did act as a moderately efficient reservoir during chronic disease [58, 59].

These observations suggest 2 non-mutually exclusive modes of transmission (Fig 2). One mode is the acquisition of low numbers of parasites by uninfected sand flies feeding on individuals with low parasite loads and mild or asymptomatic chronic disease. These flies in turn have infections with low parasite numbers and low frequencies of metacyclic promastigotes, and transmit less severe disease. This "mild/asymptomatic" mode of transmission may help explain the maintenance of the parasite in a given population without severe clinical disease. For example, in an investigation in Bhutan, only 1 kala-azar case was detected in a village, yet

Fig 2. Two modes of sand fly transmission under the influence of dose and the biological inputs that influence them. Flies feeding on mammalian hosts with a high parasite load are infected with a high dose of parasites, generating infections with a high frequency of metacyclic promastigotes that are transmitted to a second mammalian host with high efficiency and in larger numbers, resulting in more severe disease [54]. Higher dose infections in the mammalian host result in more severe acute disease but with more complete resolution and lower parasite loads in the chronic phase. Lower dose infections result in mild acute disease but chronic moderate disease [58, 59]. High acute parasite loads act as highly efficient reservoirs for disease, while low chronic parasite loads are very poor reservoirs for disease, and chronic moderate parasite loads are moderate reservoirs for disease [58–60]. Individuals with high parasite loads are mammalian "super-spreaders" by virtue of their high reservoir potential, while sand flies with high parasite loads are sand fly "super-spreaders" by virtue of their highly efficient transmission of parasites.

35% of the surveyed residents had positive LST results, and the age-prevalence curve strongly suggested chronic low-level transmission over many years [61].

The second mode of transmission occurs when sand flies feed on a heavily infected individual and develop an infection with high parasite numbers and high frequencies of metacyclic promastigotes. When these flies feed on a second host, they transmit a larger number of parasites, causing a more acute and severe disease. On an individual level, the transition from a mild/asymptomatic transmission event to severe/symptomatic transmission may be modulated, for example, when a mammalian host develops severe disease despite a low-dose inoculum due to host factors such as immune status, nutrition, and genetics [62]. In the sand fly host, individual flies infected with a low-dose inoculum may on occasion develop more robust and transmissible infections due to sand fly host factors such as microbiota or sand fly immunity. Alternatively, a poorly infected sand fly may transmit a larger dose of parasites, something that has been shown to occur experimentally, albeit rarely [59]. High-dose transmission by a poorly infected fly is likely related to sand fly feeding behavior, as described below, for the "blocked fly phenotype."

Effects of exposure to sand fly saliva on mammalian hosts

Sand flies probe the skin and lacerate the upper dermal capillaries, forming a pool of blood, and continuously inject saliva into the wound to prevent clotting [63, 64]. Sand fly salivary

gland homogenate has been shown to exacerbate experimental leishmaniasis in naïve animals when co-inoculated with parasites or confer protection in animals exposed to infected sand fly bites or *Leishmania* plus salivary proteins [65–67]. In experimental models, the most protective salivary proteins induced a delayed type hypersensitivity (DTH) response in skin as early as 6 hours post bite, skewed towards a pro-inflammatory (Th1) phenotype [66, 68]. This focal cellular immunity is thought to act against the earliest stages of infection, reducing parasite survival and ability to initiate disease. Salivary proteins that induce such responses protect against a variety of *Leishmania* species in animal models [63], although the specific mechanism has yet to be fully elucidated. In humans, DTH has also been shown to occur in individuals exposed to uninfected sand flies [69, 70]. Despite this, endemic transmission continues in populations that are frequently bitten by sand flies, suggesting a lack of protection in humans, decay of immune responses between transmission seasons [71], or variable effects of salivary components [72–74].

Leishmania adapt and manipulate their sand fly hosts for efficient transmission

The dose and origin of the infecting parasites influence the course of the infection. Transmission can result from either direct inoculation of parasites in the proboscis or foregut, or regurgitation from a more posterior station in the midgut. As *Leishmania* undergo transformations in the sand fly gut, they produce promastigote secretory gel (PSG) consisting of parasite proteophosphoglycans, including filamentous proteophosphoglycan (fPPG) and secreted acid phosphatases [75–77]. PSG plug formation occurs during metacyclogenesis, when the parasites have succeeded in colonizing the anterior midgut and stomodeal valve [76, 78]. For *L. mexicana* in *Lutzomyia longipalpis* [78, 79], the infected midgut can expand to 3 times its original volume, forcing the stomodeal valve permanently open [76]. Damage to the valve may promote reflux of parasites into the skin during blood feeding [80] and *Leishmania* secrete chitinases to further weaken the valve [81]. The combination of PSG and chitinase secretion results in gut distortion and valve dysfunction, causing more persistent feeding attempts and resulting in larger lesions and parasite burdens. In addition, sand flies with the highest number of metacyclic promastigotes have the most fPPG in their midguts and are the most persistent in attempting to feed (the "blocked sand fly" phenotype) [82]. Thus, PSG appears to be the manipulator molecule for *Leishmania*, interfering with blood flow and vector perception of blood intake. Increased blood-feeding persistence is also associated with a higher probability of feeding on multiple hosts in close proximity [76].

Scantily infected flies with enough PSG near the stomodeal valve could disgorge most of their infection in 1 bite and provide exceptions to the relationship between fly infection intensity and onward transmission. In 1 experiment, a single *L. major*-infected *Phlebotomus papatasi* with a low infection transmitted 14% of its prefeed load, which was comparable to the high-dose transmitters in the same study [59]. This combination of a low dose of parasites with a high dose of infection-enhancing PSG, exacerbated by modified feeding behavior, may favor acute and severe disease in a host and tip the balance towards the symptomatic/severe form of transmission. If proven, these flies might be considered "super-spreaders" and have epidemiological significance.

PSG has also been shown to enhance *Leishmania* infection in the skin [59] and viscera [83] of mammalian hosts. Interestingly, cutaneous lesions developed at the inoculation site when *L. infantum* was coinjected with PSG, suggesting that PSG can promote the survival and persistence of *Leishmania* in skin, irrespective of its cutaneous or visceralizing clinical phenotype,

and it may contribute towards the onward transmissibility to other sand flies, as the amastigote dose significantly influences vectorial capacity.

Implications for transmission and control

The existence of mammalian and sand fly "super-spreaders" provides a biological basis for the spatial and temporal clustering of clinical leishmanial disease. Blood-feeding vectors, including sand flies, are not uniformly distributed within or between susceptible host species [84, 85]. Nonhomogeneous mixing of vectors and hosts usually results in higher transmission rates and greater infection persistence compared to homogeneously mixed populations [84–87]. In nature, infections of wildlife hosts of *Leishmania* are typically subclinical and benign, with varying degrees of tissue tropism, parasite loads, and infectiousness to sand flies, even when hosts live in close association with heavily infected vector populations [53, 88]. Such observations highlight the specificity of host—parasite—vector relationships and the broad spectrum of possible modes of *Leishmania* maintenance and transmission.

Interventions need to have sufficient geographic coverage to include transmission hot spots, especially as current field diagnostic tools do not distinguish highly infectious vectors or hosts from those that are not infectious [38]. To interrupt transmission, specific rapid tests that identify infectiousness are needed. If an intervention suitable for asymptomatically infected individuals were developed, a similar human test would be needed to enable appropriate targeting to those contributing to ongoing transmission. Interventions must be flexible enough to take the dynamics of the disease into account as the leishmaniasis transmission varies spatially and over the course of an epidemic cycle. Interventions that reduce sand fly densities in the absence of elimination could interfere with potential saliva-conferred partial immunity against *Leishmania* [63–70]. Such reductions could also affect vector aggregation dynamics, causing a shift in the attractiveness of sand fly leks from dead-end hosts to humans and animal reservoirs. In turn, this could affect sand fly density-dependent blood-feeding success [89]: incomplete feeding or interrupted probing may lead to multiple bites, promoting transmission within spatially defined host populations [85, 86]. Certainly, sand fly blood-feeding behavior will determine the efficacies of indoor residual spraying, topical insecticides, and bed nets [90, 91]. Alterations in biting behavior affecting the suitability of these methods could be induced by insecticide pressure, as observed in mosquitoes [92, 93], although no such studies have been conducted in sand flies. A deeper understanding of both sand fly and host biology and behavior is therefore essential to ensuring effectiveness of vector interventions and avoiding unintended counterproductive consequences.

Acknowledgments

References

1. Singh OP, Hasker E, Boelaert M, Sundar S. Elimination of visceral leishmaniasis on the Indian subcontinent. The Lancet infectious diseases. 2016; 16(12):e304–e9. Epub 2016/10/04. https://doi.org/10.1016/S1473-3099(16)30140-2 PMID: 27692643

2. Jeronimo SMB, de Queiroz Sousa A, Pearson RD. Leishmaniasis. In: Guerrant RL, Walker DH, Weller PF, editors. Tropical infectious diseases: principles, pathogens and practice. 3rd ed. Edinburgh, Scotland: Saunders Elsevier; 2011. p. 696–706.

3. Napier LE, Krishnan KV. A theory of the aetiology and epidemiology of kala-azar in India. Indian Medical Gazette. 1931; 66(11):603–9.

4. Alvar J, Velez ID, Bern C, Herrero M, Desjeux P, Cano J, et al. Leishmaniasis worldwide and global estimates of its incidence. PLoS ONE. 2012; 7(5):e35671. Epub 2012/06/14. https://doi.org/10.1371/journal.pone.0035671 PMID: 22693548

5. Dye C, Wolpert DM. Earthquakes, influenza and cycles of Indian kala-azar. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1988; 82(6):843–50. PMID: 3256984

6. Islam S, Kenah E, Bhuiyan MA, Rahman KM, Goodhew B, Ghalib CM, et al. Clinical and immunological aspects of post-kala-azar dermal leishmaniasis in Bangladesh. The American journal of tropical medicine and hygiene. 2013; 89(2):345–53. Epub 2013/07/03. https://doi.org/10.4269/ajtmh.12-0711 PMID: 23817330

7. Malaviya P, Picado A, Singh SP, Hasker E, Singh RP, Boelaert M, et al. Visceral leishmaniasis in Muzaffarpur district, Bihar, India from 1990 to 2008. PLoS ONE. 2011; 6(3):e14751. Epub 2011/03/12. https://doi.org/10.1371/journal.pone.0014751 PMID: 21394195

8. Werneck GL, Rodrigues L, Santos MV, Araujo IB, Moura LS, Lima SS, et al. The burden of Leishmania chagasi infection during an urban outbreak of visceral leishmaniasis in Brazil. Acta tropica. 2002; 83 (1):13–8. PMID: 12062788

9. Elnaiem DE, Schorscher J, Bendall A, Obsomer V, Osman ME, Mekkawi AM, et al. Risk mapping of visceral leishmaniasis: the role of local variation in rainfall and altitude on the presence and incidence of kala-azar in eastern Sudan. The American journal of tropical medicine and hygiene. 2003; 68(1):10–7. PMID: 12556141

10. Bern C, Amann J, Haque R, Chowdhury R, Ali M, Kurkjian KM, et al. Loss of leishmanin skin test antigen sensitivity and potency in a longitudinal study of visceral leishmaniasis in Bangladesh. The American journal of tropical medicine and hygiene. 2006; 75(4):744–8. PMID: 17038705

11. Khalil EA, Zijlstra EE, Kager PA, El Hassan AM. Epidemiology and clinical manifestations of *Leishmania donovani* infection in two villages in an endemic area in eastern Sudan. Tropical medicine & international health: TM & IH. 2002; 7(1):35–44.

12. Davies CR, Mazloumi Gavgani AS. Age, acquired immunity and the risk of visceral leishmaniasis: a prospective study in Iran. Parasitology. 1999; 119(Pt 3):247–57.

13. Bucheton B, Kheir MM, El-Safi SH, Hammad A, Mergani A, Mary C, et al. The interplay between environmental and host factors during an outbreak of visceral leishmaniasis in eastern Sudan. Microbes and infection / Institut Pasteur. 2002; 4(14):1449–57.

14. Alvar J, Bashaye S, Argaw D, Cruz I, Aparicio P, Kassa A, et al. Kala-azar outbreak in Libo Kemkem, Ethiopia: epidemiologic and parasitologic assessment. The American journal of tropical medicine and hygiene. 2007; 77(2):275–82. PMID: 17690399

15. Bora D. Epidemiology of visceral leishmaniasis in India. Natl Med J India. 1999; 12(2):62–8. PMID: 10416321

16. Elias M, Rahman AJ, Khan NI. Visceral leishmaniasis and its control in Bangladesh. Bull World Health Organ. 1989; 67(1):43–9. PMID: 2650901

17. Ostyn B, Vanlerberghe V, Picado A, Dinesh DS, Sundar S, Chappuis F, et al. Vector control by insecticide-treated nets in the fight against visceral leishmaniasis in the Indian subcontinent, what is the evidence? Tropical medicine & international health: TM & IH. 2008; 13(8):1073–85.

18. World Health Organization. Leishmaniasis: Country profiles Geneva, Switzerland2014 [cited 2017 02/28/2017]. http://www.who.int/leishmaniasis/burden/Country_profiles/en/.

19. Bhunia GS, Kesari S, Chatterjee N, Kumar V, Das P. Spatial and temporal variation and hotspot detection of kala-azar disease in Vaishali district (Bihar), India. BMC Infect Dis. 2013; 13:64. Epub 2013/02/05. https://doi.org/10.1186/1471-2334-13-64 PMID: 23375077

20. Werneck GL, Costa CH, Walker AM, David JR, Wand M, Maguire JH. The urban spread of visceral leishmaniasis: clues from spatial analysis. Epidemiology. 2002; 13(3):364–7. PMID: 11964941

21. Bern C, Hightower AW, Chowdhury R, Ali M, Amann J, Wagatsuma Y, et al. Risk factors for kala-azar in Bangladesh. Emerg Infect Dis. 2005; 11(5):655–62. Epub 2005/05/14. https://doi.org/10.3201/eid1105.040718 PMID: 15890115

22. Ashford RW, Kohestany KA, Karimzad MA. Cutaneous leishmaniasis in Kabul: observations on a 'prolonged epidemic'. Annals of tropical medicine and parasitology. 1992; 86(4):361–71. PMID: 1463356

23. Evans TG, Teixeira MJ, McAuliffe IT, Vasconcelos I, Vasconcelos AW, Sousa Ade A, et al. Epidemiology of visceral leishmaniasis in northeast Brazil. The Journal of infectious diseases. 1992; 166 (5):1124–32. PMID: 1402024

24. Molina R, Jimenez MI, Cruz I, Iriso A, Martin-Martin I, Sevillano O, et al. The hare (Lepus granatensis) as potential sylvatic reservoir of Leishmania infantum in Spain. Veterinary parasitology. 2012; 190(1–2):268–71. Epub 2012/06/09. https://doi.org/10.1016/j.vetpar.2012.05.006 PMID: 22677135

25. Bray DP, Carter V, Alves GB, Brazil RP, Bandi KK, Hamilton JG. Synthetic sex pheromone in a long-lasting lure attracts the visceral leishmaniasis vector, Lutzomyia longipalpis, for up to 12 weeks in Brazil. PLoS Negl Trop Dis. 2014; 8(3):e2723. Epub 2014/03/22. https://doi.org/10.1371/journal.pntd.0002723 PMID: 24651528

26. Bray DP, Yaman K, Underhilll BA, Mitchell F, Carter V, Hamilton JG. Multi-modal analysis of courtship behaviour in the old world leishmaniasis vector Phlebotomus argentipes. PLoS Negl Trop Dis. 2014; 8 (12):e3316. Epub 2014/12/05. https://doi.org/10.1371/journal.pntd.0003316 PMID: 25474027

27. Lane RP, Pile MM, Amerasinghe FP. Anthropophagy and aggregation behaviour of the sandfly Phlebotomus argentipes in Sri Lanka. Medical and veterinary entomology. 1990; 4(1):79–88. PMID: 2132972

28. Bray DP, Ward RD, Hamilton JGC. The chemical ecology of sandflies. In: Takken W, Knols BGJ, editors. Ecology and control of vector-borne diseases. 2. Wageningen, Netherlands: Wageningen Academic Publishers; 2010. p. 203–16.

29. Dye C, Guy MW, Elkins DB, Wilkes TJ, Killick-Kendrick R. The life expectancy of phlebotomine sandflies: first field estimates from southern France. Medical and veterinary entomology. 1987; 1(4):417–25. Epub 1987/10/01. PMID: 2979558

30. Christophers S, Shortt H, Barraud P. The development of the parasite of Indian kala-azar in the sandfly Phlebotomus argentipes Annandale and Brunetti. The Indian journal of medical research. 1924; 12:605–7.

31. Uranw S, Ostyn B, Rijal A, Devkota S, Khanal B, Menten J, et al. Post-kala-azar dermal leishmaniasis in Nepal: a retrospective cohort study (2000–2010). PLoS Negl Trop Dis. 2011; 5(12):e1433. Epub 2011/12/30. https://doi.org/10.1371/journal.pntd.0001433 PMID: 22206030

32. Zijlstra EE, el-Hassan AM, Ismael A, Ghalib HW. Endemic kala-azar in eastern Sudan: a longitudinal study on the incidence of clinical and subclinical infection and post-kala-azar dermal leishmaniasis. The American journal of tropical medicine and hygiene. 1994; 51(6):826–36. PMID: 7810819

33. Addy M, Nandy A. Ten years of kala-azar in west Bengal, Part I. Did post-kala-azar dermal leishmaniasis initiate the outbreak in 24-Parganas? Bull World Health Organ. 1992; 70(3):341–6. PMID: 1638662

34. Napier L, Smith R, Das-Gupta C, Mukerji S. The infection of Phlebotomus argentipes from dermal leishmanial lesions. The Indian journal of medical research. 1933; 21:173–7.

35. Zijlstra EE, Musa AM, Khalil EA, el-Hassan IM, el-Hassan AM. Post-kala-azar dermal leishmaniasis. The Lancet infectious diseases. 2003; 3(2):87–98. PMID: 12560194

36. Molina R, Ghosh D, Carrillo E, Monnerat S, Bern C, Mondal D, et al. Infectivity of Post-Kala-azar Dermal Leishmaniasis patients to sand flies: revisiting a Proof of Concept in the context of the Kala-azar Elimination Program in the Indian subcontinent. Clin Infect Dis. 2017; 65(1):150–3. Epub 18 May 2017. https://doi.org/10.1093/cid/cix245 PMID: 28520851

37. Courtenay O, Carson C, Calvo-Bado L, Garcez L, Quinnell R. Heterogeneities in Leishmania infantum infection: using skin parasite burdens to identify highly infectious dogs. PLoS Negl Trop Dis. 2014; 8(1): e2583. https://doi.org/10.1371/journal.pntd.0002583 PMID: 24416460

38. Courtenay O, Quinnell RJ, Garcez LM, Shaw JJ, Dye C. Infectiousness in a cohort of brazilian dogs: why culling fails to control visceral leishmaniasis in areas of high transmission. The Journal of infectious diseases. 2002; 186(9):1314–20. https://doi.org/10.1086/344312 PMID: 12402201

39. Travi BL, Tabares CJ, Cadena H, Ferro C, Osorio Y. Canine visceral leishmaniasis in Colombia: relationship between clinical and parasitologic status and infectivity for sand flies. The American journal of tropical medicine and hygiene. 2001; 64(3–4):119–24. PMID: 11442205

40. Molina R, Amela C, Nieto J, San-Andres M, Gonzalez F, Castillo JA, et al. Infectivity of dogs naturally infected with Leishmania infantum to colonized Phlebotomus perniciosus. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1994; 88(4):491–3. Epub 1994/07/01. PMID: 7570854

41. Stauch A, Sarkar RR, Picado A, Ostyn B, Sundar S, Rijal S, et al. Visceral leishmaniasis in the Indian subcontinent: modelling epidemiology and control. PLoS Negl Trop Dis. 2011; 5(11):e1405. Epub 2011/12/06. https://doi.org/10.1371/journal.pntd.0001405 PMID: 22140589

42. Ali A, Ashford RW. Visceral leishmaniasis in Ethiopia. IV. Prevalence, incidence and relation of infection to disease in an endemic area. Annals of tropical medicine and parasitology. 1994; 88(3):289–93. PMID: 7944674

43. Bern C, Haque R, Chowdhury R, Ali M, Kurkjian KM, Vaz L, et al. The epidemiology of visceral leishmaniasis and asymptomatic leishmanial infection in a highly endemic Bangladeshi village. The American journal of tropical medicine and hygiene. 2007; 76(5):909–14. PMID: 17488915

44. Hasker E, Malaviya P, Gidwani K, Picado A, Ostyn B, Kansal S, et al. Strong association between serological status and probability of progression to clinical visceral leishmaniasis in prospective cohort studies in India and Nepal. PLoS Negl Trop Dis. 2014; 8(1):e2657. Epub 2014/01/28. https://doi.org/10.1371/journal.pntd.0002657 PMID: 24466361

45. Hirve S, Boelaert M, Matlashewski G, Mondal D, Arana B, Kroeger A, et al. Transmission Dynamics of Visceral Leishmaniasis in the Indian Subcontinent—A Systematic Literature Review. PLoS Negl Trop Dis. 2016; 10(8):e0004896. Epub 2016/08/05. https://doi.org/10.1371/journal.pntd.0004896 PMID: 27490264

46. Matlashewski G, Arana B, Kroeger A, Battacharya S, Sundar S, Das P, et al. Visceral leishmaniasis: elimination with existing interventions. The Lancet infectious diseases. 2011; 11(4):322–5. Epub 2011/04/02. https://doi.org/10.1016/S1473-3099(10)70320-0 PMID: 21453873

47. Singh OP, Hasker E, Sacks D, Boelaert M, Sundar S. Asymptomatic Leishmania infection: a new challenge for Leishmania control. Clin Infect Dis. 2014; 58(10):1424–9. Epub 2014/03/04. https://doi.org/10.1093/cid/ciu102 PMID: 24585564

48. Sudarshan M, Sundar S. Parasite load estimation by qPCR differentiates between asymptomatic and symptomatic infection in Indian visceral leishmaniasis. Diagnostic microbiology and infectious disease. 2014; 80(1):40–2. Epub 2014/07/16. https://doi.org/10.1016/j.diagmicrobio.2014.01.031 PMID: 25023070

49. Sudarshan M, Singh T, Chakravarty J, Sundar S. A Correlative Study of Splenic Parasite Score and Peripheral Blood Parasite Load Estimation by Quantitative PCR in Visceral Leishmaniasis. Journal of clinical microbiology. 2015; 53(12):3905–7. Epub 2015/09/25. https://doi.org/10.1128/JCM.01465-15 PMID: 26400788

50. Sudarshan M, Singh T, Singh AK, Chourasia A, Singh B, Wilson ME, et al. Quantitative PCR in epidemiology for early detection of visceral leishmaniasis cases in India. PLoS Negl Trop Dis. 2014; 8(12):e3366. Epub 2014/12/17. https://doi.org/10.1371/journal.pntd.0003366 PMID: 25503103

51. Quinnell RJ, Courtenay O. Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. Parasitology. 2009:1–20.

52. Courtenay O, Quinnell RJ, Garcez LM, Dye C. Low infectiousness of a wildlife host of Leishmania infantum: the crab-eating fox is not important for transmission. Parasitology. 2002; 125(Pt 5):407–14. PMID: 12458824

53. Andrade MS, Courtenay O, Brito ME, Carvalho FG, Carvalho AW, Soares F, et al. Infectiousness of Sylvatic and Synanthropic Small Rodents Implicates a Multi-host Reservoir of Leishmania (Viannia) braziliensis. PLoS Negl Trop Dis. 2015; 9(10):e0004137. Epub 2015/10/09. https://doi.org/10.1371/journal.pntd.0004137 PMID: 26448187

54. Stamper LW, Patrick RL, Fay MP, Lawyer PG, Elnaiem DE, Secundino N, et al. Infection parameters in the sand fly vector that predict transmission of Leishmania major. PLoS Negl Trop Dis. 2011; 5(8):e1288. Epub 2011/09/03. https://doi.org/10.1371/journal.pntd.0001288 PMID: 21886852

55. Kelly PH, Bahr SM, Serafim TD, Ajami NJ, Petrosino JF, Meneses C, et al. The Gut Microbiome of the Vector Lutzomyia longipalpis Is Essential for Survival of Leishmania infantum. mBio. 2017; 8(1). Epub 2017/01/18. https://doi.org/10.1128/mBio.01121-16 PMID: 28096483

56. Louradour I, Monteiro CC, Inbar E, Ghosh K, Merkhofer R, Lawyer P, et al. The midgut microbiota plays an essential role in sand fly vector competence for Leishmania major. Cellular microbiology. 2017. Epub 2017/06/06. https://doi.org/10.1111/cmi.12755 PMID: 28580630

57. Sant'Anna MR, Diaz-Albiter H, Aguiar-Martins K, Al Salem WS, Cavalcante RR, Dillon VM, et al. Colonisation resistance in the sand fly gut: Leishmania protects Lutzomyia longipalpis from bacterial infection. Parasites & vectors. 2014; 7:329. Epub 2014/07/24. https://doi.org/10.1186/1756-3305-7-329 PMID: 25051919

58. Lira R, Doherty M, Modi G, Sacks D. Evolution of lesion formation, parasitic load, immune response, and reservoir potential in C57BL/6 mice following high- and low-dose challenge with Leishmania major. Infection and immunity. 2000; 68(9):5176–82. Epub 2000/08/19. PMID: 10948141

59. Kimblin N, Peters N, Debrabant A, Secundino N, Egen J, Lawyer P, et al. Quantification of the infectious dose of Leishmania major transmitted to the skin by single sand flies. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105(29):10125–30. Epub 2008/07/16. https://doi.org/10.1073/pnas.0802331105 PMID: 18626016

60. Mendez S, Gurunathan S, Kamhawi S, Belkaid Y, Moga MA, Skeiky YA, et al. The potency and durability of DNA- and protein-based vaccines against Leishmania major evaluated using low-dose, intradermal challenge. J Immunol. 2001; 166(8):5122–8. Epub 2001/04/06. PMID: 11290794

61. Yangzom T, Cruz I, Bern C, Argaw D, den Boer M, Velez ID, et al. Endemic transmission of visceral leishmaniasis in Bhutan. The American journal of tropical medicine and hygiene. 2012; 87(6):1028–37. Epub 2012/10/24. https://doi.org/10.4269/ajtmh.2012.12-0211 PMID: 23091191

62. LeishGEN Consortium, Wellcome Trust Case Control Consortium, Fakiola M, Strange A, Cordell H. Common variants in the HLA-DRB1-HLA-DQA1 HLA class II region are associated with susceptibility to visceral leishmaniasis. Nat Genet. 2013; 45(2):208–13. https://doi.org/10.1038/ng.2518 PMID: 23291585

63. Gomes R, Oliveira F. The immune response to sand fly salivary proteins and its influence on leishmania immunity. Frontiers in immunology. 2012; 3:110. Epub 2012/05/18. https://doi.org/10.3389/fimmu.2012.00110 PMID: 22593758

64. Ribeiro JM. Role of saliva in blood-feeding by arthropods. Annual review of entomology. 1987; 32:463–78. Epub 1987/01/01. https://doi.org/10.1146/annurev.en.32.010187.002335 PMID: 2880553

65. Kamhawi S. The biological and immunomodulatory properties of sand fly saliva and its role in the establishment of Leishmania infections. Microbes and infection / Institut Pasteur. 2000; 2(14):1765–73. Epub 2001/01/04.

66. Kamhawi S, Belkaid Y, Modi G, Rowton E, Sacks D. Protection against cutaneous leishmaniasis resulting from bites of uninfected sand flies. Science. 2000; 290(5495):1351–4. Epub 2000/11/18. PMID: 11082061

67. Titus RG, Ribeiro JM. Salivary gland lysates from the sand fly Lutzomyia longipalpis enhance Leishmania infectivity. Science. 1988; 239(4845):1306–8. Epub 1988/03/11. PMID: 3344436

68. Oliveira F, Kamhawi S, Seitz AE, Pham VM, Guigal PM, Fischer L, et al. From transcriptome to immunome: identification of DTH inducing proteins from a Phlebotomus ariasi salivary gland cDNA library. Vaccine. 2006; 24(3):374–90. Epub 2005/09/13. https://doi.org/10.1016/j.vaccine.2005.07.085 PMID: 16154670

69. Belkaid Y, Valenzuela JG, Kamhawi S, Rowton E, Sacks DL, Ribeiro JM. Delayed-type hypersensitivity to Phlebotomus papatasi sand fly bite: An adaptive response induced by the fly? Proceedings of the National Academy of Sciences of the United States of America. 2000; 97(12):6704–9. Epub 2000/06/07. PMID: 10841567

70. Vinhas V, Andrade BB, Paes F, Bomura A, Clarencio J, Miranda JC, et al. Human anti-saliva immune response following experimental exposure to the visceral leishmaniasis vector, Lutzomyia longipalpis. European journal of immunology. 2007; 37(11):3111–21. Epub 2007/10/16. https://doi.org/10.1002/eji.200737431 PMID: 17935072

71. Rohousova I, Hostomska J, Vlkova M, Kobets T, Lipoldova M, Volf P. The protective effect against Leishmania infection conferred by sand fly bites is limited to short-term exposure. International journal for parasitology. 2011; 41(5):481–5. Epub 2011/02/12. https://doi.org/10.1016/j.ijpara.2011.01.003 PMID: 21310158

72. Rohousova I, Ozensoy S, Ozbel Y, Volf P. Detection of species-specific antibody response of humans and mice bitten by sand flies. Parasitology. 2005; 130(Pt 5):493–9. Epub 2005/07/05. PMID: 15991492

73. Marzouki S, Ben Ahmed M, Boussoffara T, Abdeladhim M, Ben Aleya-Bouafif N, Namane A, et al. Characterization of the antibody response to the saliva of Phlebotomus papatasi in people living in endemic areas of cutaneous leishmaniasis. The American journal of tropical medicine and hygiene. 2011; 84(5):653–61. Epub 2011/05/05. https://doi.org/10.4269/ajtmh.2011.10-0598 PMID: 21540371

74. de Moura TR, Oliveira F, Novais FO, Miranda JC, Clarencio J, Follador I, et al. Enhanced Leishmania braziliensis infection following pre-exposure to sandfly saliva. PLoS Negl Trop Dis. 2007; 1(2):e84. Epub 2007/12/07. https://doi.org/10.1371/journal.pntd.0000084 PMID: 18060088

75. Bates PA. Transmission of Leishmania metacyclic promastigotes by phlebotomine sand flies. International journal for parasitology. 2007; 37(10):1097–106. Epub 2007/05/23. https://doi.org/10.1016/j.ijpara.2007.04.003 PMID: 17517415

76. Rogers ME, Chance ML, Bates PA. The role of promastigote secretory gel in the origin and transmission of the infective stage of *Leishmania mexicana* by the sandfly *Lutzomyia longipalpis*. Parasitology. 2002; 124(Pt 5):495–507. Epub 2002/06/07. PMID: 12049412

77. Rogers ME. The role of leishmania proteophosphoglycans in sand fly transmission and infection of the Mammalian host. Frontiers in microbiology. 2012; 3:223. Epub 2012/07/04. https://doi.org/10.3389/fmicb.2012.00223 PMID: 22754550

78. Rogers ME, Ilg T, Nikolaev AV, Ferguson MA, Bates PA. Transmission of cutaneous leishmaniasis by sand flies is enhanced by regurgitation of fPPG. Nature. 2004; 430(6998):463–7. Epub 2004/07/23. https://doi.org/10.1038/nature02675 PMID: 15269771

79. Rogers M, Kropf P, Choi BS, Dillon R, Podinovskaia M, Bates P, et al. Proteophosophoglycans regurgitated by Leishmania-infected sand flies target the L-arginine metabolism of host macrophages to promote parasite survival. PLoS Pathog. 2009; 5(8):e1000555. Epub 2009/08/22. https://doi.org/10.1371/journal.ppat.1000555 PMID: 19696894

80. Schlein Y, Jacobson RL, Messer G. Leishmania infections damage the feeding mechanism of the sandfly vector and implement parasite transmission by bite. Proceedings of the National Academy of Sciences of the United States of America. 1992; 89(20):9944–8. Epub 1992/10/15. PMID: 1409724

81. Rogers ME, Hajmova M, Joshi MB, Sadlova J, Dwyer DM, Volf P, et al. *Leishmania* chitinase facilitates colonization of sand fly vectors and enhances transmission to mice. Cellular microbiology. 2008; 10(6):1363–72. Epub 2008/02/21. https://doi.org/10.1111/j.1462-5822.2008.01132.x PMID: 18284631

82. Rogers ME, Bates PA. *Leishmania* manipulation of sand fly feeding behavior results in enhanced transmission. PLoS Pathog. 2007; 3(6):e91. Epub 2007/07/03. https://doi.org/10.1371/journal.ppat.0030091 PMID: 17604451

83. Rogers ME, Corware K, Muller I, Bates PA. *Leishmania infantum* proteophosphoglycans regurgitated by the bite of its natural sand fly vector, *Lutzomyia longipalpis*, promote parasite establishment in mouse skin and skin-distant tissues. Microbes and infection / Institut Pasteur. 2010; 12(11):875–9. Epub 2010/06/22. https://doi.org/10.1016/j.micinf.2010.05.014 PMID: 20561596

84. Woolhouse ME, Dye C, Etard JF, Smith T, Charlwood JD, Garnett GP, et al. Heterogeneities in the transmission of infectious agents: implications for the design of control programs. Proceedings of the National Academy of Sciences of the United States of America. 1997; 94(1):338–42. Epub 1997/01/07. PMID: 8990210

85. Kelly DW, Thompson CE. Epidemiology and optimal foraging: modelling the ideal free distribution of insect vectors. Parasitology. 2000; 120 (Pt 3):319–27. Epub 2000/04/12.

86. Dye C, Hasibeder G. Population dynamics of mosquito-borne disease: effects of flies which bite some people more frequently than others. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1986; 80(1):69–77. Epub 1986/01/01. PMID: 3727001

87. Hasibeder G, Dye C. Population dynamics of mosquito-borne disease: persistence in a completely heterogeneous environment. Theoretical population biology. 1988; 33(1):31–53. Epub 1988/02/01. PMID: 2897726

88. Yaghoobi-Ershadi M. Phlebotomine Sand Flies (Diptera: Psychodidae) in Iran and their Role on Leishmania Transmission. Journal of arthropod-borne diseases. 2012; 6(1):1–17. Epub 2013/01/08. PMID: 23293774

89. Kelly DW, Dye C. Pheromones, kairomones and the aggregation dynamics of the sandfly *Lutzomyia longipalpis* Animal Behaviour. 1997; 53(4):721–31.

90. Courtenay O, Gillingwater K, Gomes PA, Garcez LM, Davies CR. Deltamethrin-impregnated bednets reduce human landing rates of sandfly vector *Lutzomyia longipalpis* in Amazon households. Medical and veterinary entomology. 2007; 21(2):168–76. https://doi.org/10.1111/j.1365-2915.2007.00678.x PMID: 17550436

91. Picado A, Singh SP, Rijal S, Sundar S, Ostyn B, Chappuis F, et al. Longlasting insecticidal nets for prevention of Leishmania donovani infection in India and Nepal: paired cluster randomised trial. BMJ. 2010; 341:c6760. Epub 2010/12/31. https://doi.org/10.1136/bmj.c6760 PMID: 21190965

92. Reddy MR, Overgaard HJ, Abaga S, Reddy VP, Caccone A, Kiszewski AE, et al. Outdoor host seeking behaviour of Anopheles gambiae mosquitoes following initiation of malaria vector control on Bioko Island, Equatorial Guinea. Malaria journal. 2011; 10:184. Epub 2011/07/09. https://doi.org/10.1186/1475-2875-10-184 PMID: 21736750

93. Moiroux N, Gomez MB, Pennetier C, Elanga E, Djenontin A, Chandre F, et al. Changes in Anopheles funestus biting behavior following universal coverage of long-lasting insecticidal nets in Benin. The Journal of infectious diseases. 2012; 206(10):1622–9. Epub 2012/09/12. https://doi.org/10.1093/infdis/jis565 PMID: 22966127