Pathway to a controlled human infection model for *Leishmania major*

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Abstract

Leishmaniasis is a neglected tropical disease that disproportionately affects those afflicted by malnutrition and poverty. The insect vector, the sand fly, is increasingly found in temperate climates, due to the effects of climate change. There is a wide spectrum of clinical disease, from visceral leishmaniasis which affects internal organs causing hepatosplenomegaly and immune dysregulation, to cutaneous leishmaniasis which can causing disfiguring skin lesions. The particular disease that an individual exhibits is dependent upon the species of *Leishmania* involved, but also many host-related factors. The sand fly has been shown to be integral to not only disease spread, but also disease initiation with components from the sand fly salivary gland emitted during biting. These sand fly salivary gland proteins assist the parasite in evading the host immune defences to allow replication and infection to occur. There is a high degree of genetic homogeneity between *Leishmania* species, and so research focusing on one species of *Leishmania* can help in finding new treatments and control strategies for the whole genus. There are no current vaccines for leishmaniasis licensed for human use, and limited treatment options, all of which now have shown evidence of resistance. One method to accelerate vaccine candidate selection at an early stage of development is the use of controlled human infection models (CHIMs). These CHIMs have been used for many infectious diseases, including parasitic, bacterial and viral pathogens, as a measured process to assess novel therapeutic agents. This thesis describes the key steps taken in developing a novel sand fly initiated CHIM for *Leishmania major*, a form of the disease which causes predominantly localised skin lesions The first step was a public involvement (PI) exercise to assess public acceptability and perception of a CHIM approach using infected sand flies. This exercise reinforced the need for clarity of information, but also safety checkpoints to allow for participant confidence in the processes. This PI group activity directly influenced the methodology of the proposed work. The next step was a study demonstrating the safety and efficacy of a protocol using infected sand fly biting on human volunteers (the FLYBITE study). Acceptability was confirmed post-study by focus group, which also impacted on future study considerations. Finally, an efficacious and safe novel CHIM for sand fly initiated cutaneous leishmaniasis on human volunteers was developed, which now has the potential to be used for emerging vaccine candidates in the development pipeline.

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Although writing this thesis has been a labour of love, perhaps this has been the most difficult section to write, not least due to the number of people that have supported me during these last years.

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I started out at York at the inception of the York Biomedical Research Institute (YBRI), and during my time here, I have worked with many colleagues who I consider friends. Thank you to all the people who I worked closely with and shared many an anecdote with! In particularly thank you to Helen Ashwin, who worked with me and offered support throughout this project, and also Becky Wiggins, who taught me many of the lab processes, and steered me away from ruining all my PBMCs! Thank you also to Liz Greensted, Naj Brown, Mohamed Osman, Katrien Van Bocxlaer, Nidhi Dey, Gulab Rani and Shoumit Dey for all your words of encouragement and collaboration. Thank you also to all the research nurses, Nicola Marshall, Siobhan and Jo Ingram, who made the clinical aspects a delight! I can't forget David Thompson for his support throughout this project.

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I hope that I continue use the knowledge and experiences I have gained here with humility towards colleagues, patients, and my family. As Baba Hardev Singh Ji once said – "Kuch bhi bano mubarak hai, pehle bus insaan bano" – whatever you achieve in life, first become a decent human being.

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5. **Parkash V**, Ashwin H, Dey S, Sadlova J, Vojtkova B, Van Bocxlaer K, Wiggins R, Prow N, Sharma Dey N, Layton AM, Jaffe CL, Schwartz E, Volf P, Lacey CJN and Kaye PM. Safety, effectiveness and skin immune response in a controlled human infection model of sand fly transmitted cutaneous leishmaniasis. ***Submitted for publication****.*

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2. Keeley AJ, **Parkash V**, Tunbridge A, Greig J, Collini P, McKane W, Tattersall RS. Anakinra in the treatment of protracted paradoxical inflammatory reactions in HIV-associated tuberculosis in the United Kingdom: a report of two cases. Int J STD AIDS. 2020 Jul;31(8):808-812. doi: 10.1177/0956462420915394. PMID: 32631210; PMCID: PMC7590809.

3. Shahin Y, Rajaram S, **Parkash V**, Wild JM, Kiely DG, Swift AJ. Patterns of thromboembolic pulmonary vascular disease in COVID-19. Pulm Circ. 2021 Jan 21;11(1):2045894020979198. doi: 10.1177/2045894020979198. PMID: 33532054; PMCID: PMC7829513.

4. RECOVERY Collaborative Group. Baricitinib in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial and updated meta-analysis. Lancet. 2022 Jul 30;400(10349):359-368. doi: 10.1016/S0140-6736(22)01109-6. Erratum in: Lancet. 2022 Oct 1;400(10358):1102. PMID: 35908569; PMCID: PMC9333998.

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1.1 Leishmaniasis

1.1.1 Background

The leishmaniases represent a diverse group of parasitic diseases caused by protozoan parasites of the genus *Leishmania.* To-date over 50 species of *Leishmania* have been discovered (Travi et al., 2018), although only 20 species belonging to two subgenera are so far associated with human disease (Mondal et al., 2019). *Leishmania sp.* are transmitted by the bite of phlebotomine sand flies, of which the *Lutzomyia* and *Phlebotomus* genera are so far associated with natural transmission to humans (Mondal et al., 2019). It has been shown that species of *Leishmania* are closely related and have a highly conserved genome, which may have implications for cross-protection after exposure (see Figure 1.1). The parasite is microscopic, at ~ 15um in length (see Figure 1.2). It is part of a broad group of parasites known as protozoa which also include parasites such as the malaria-causing *Plasmodium*. A sub-group of parasites are the hemoflagellates, which includes the genera *Trypanosoma* as well as *Leishmania.* These are so called due to the site of preponderance within human beings, typically the blood, as well as presence of flagella which aid motility.

The *Leishmania* lifecycle is complex and is partially dependent upon the vector to allow maturation and division (see Figure 1.3). The parasite-vector-host interaction is also often described as complex, with several factors influencing phenotypic differences in disease manifestation. Although the past few decades have brought with them improved understanding, several important mechanisms of disease remain to be elucidated (Colmenares et al., 2002; Cecílio, Cordeiro-da-Silva and Oliveira, 2022). Vaccines are already successfully in use for the control of canine leishmaniasis which is a significant worldwide issue. Within this setting they are effective at reducing transmission by impacting on the infection reservoir. A human vaccine, so far, remains elusive. As with many neglected tropical diseases, there is a mismatch between availability of resources for research opportunities and disease burden (Kamhawi, 2017; Fonseca, Albuquerque and Zicker, 2020). Controlled human infection models (CHIM), have been mooted as useful research adjuncts to assist in evaluating novel therapies, especially in the case of neglected tropical diseases

where research must be cost-effective and efficient at selecting vaccine candidates at early stages of development (Darton et al., 2015). Such studies involve deliberate infection of healthy participants in order to study efficacy of candidate vaccines, disease progression, and human immunopathology.

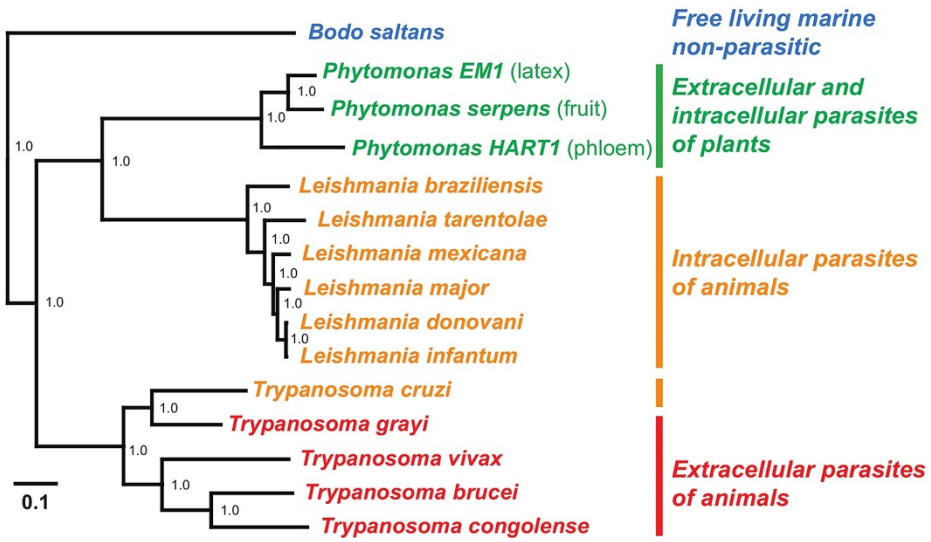

Figure 1.1: Trypanosomatid phylogenetic tree including *Leishmania* species

The trypanosomatids are a related group of unicellular predominantly parasitic organisms distinguished by presence of a flagellum and presence of a kinetoplast. From (Jaskowska et al., 2015).

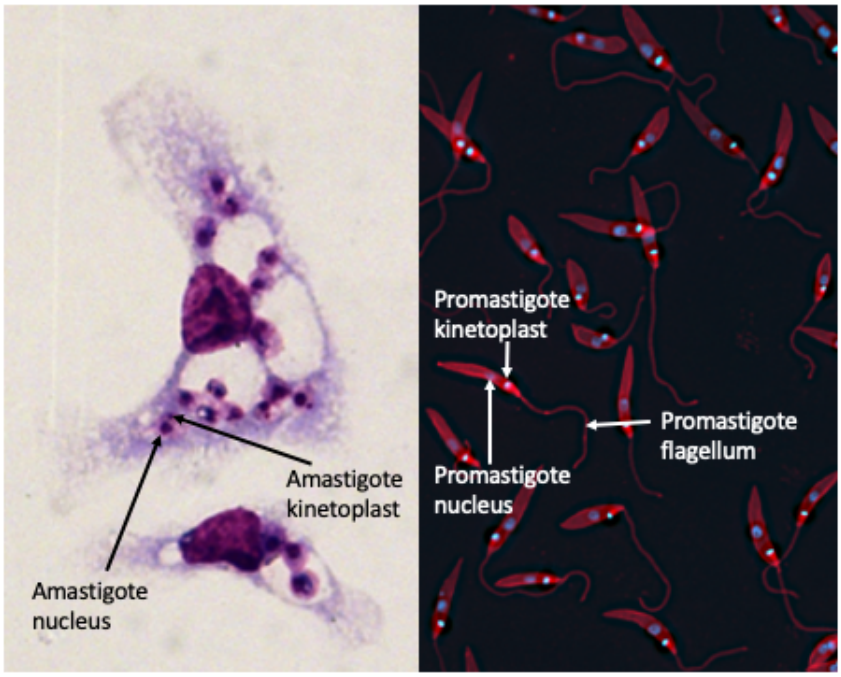

Figure 1.2: Leishmania parasite images

Left image: *Leishmania* amastigotes (mammalian form) with Giemsa stain. Right image: *Leishmania* promastigotes (sand fly form) stained with a membrane stain and DAPI fluorescent stain. Images adapted from those provided by Dr K Van Bocxlaer & Dr S Antoniou, University of York.

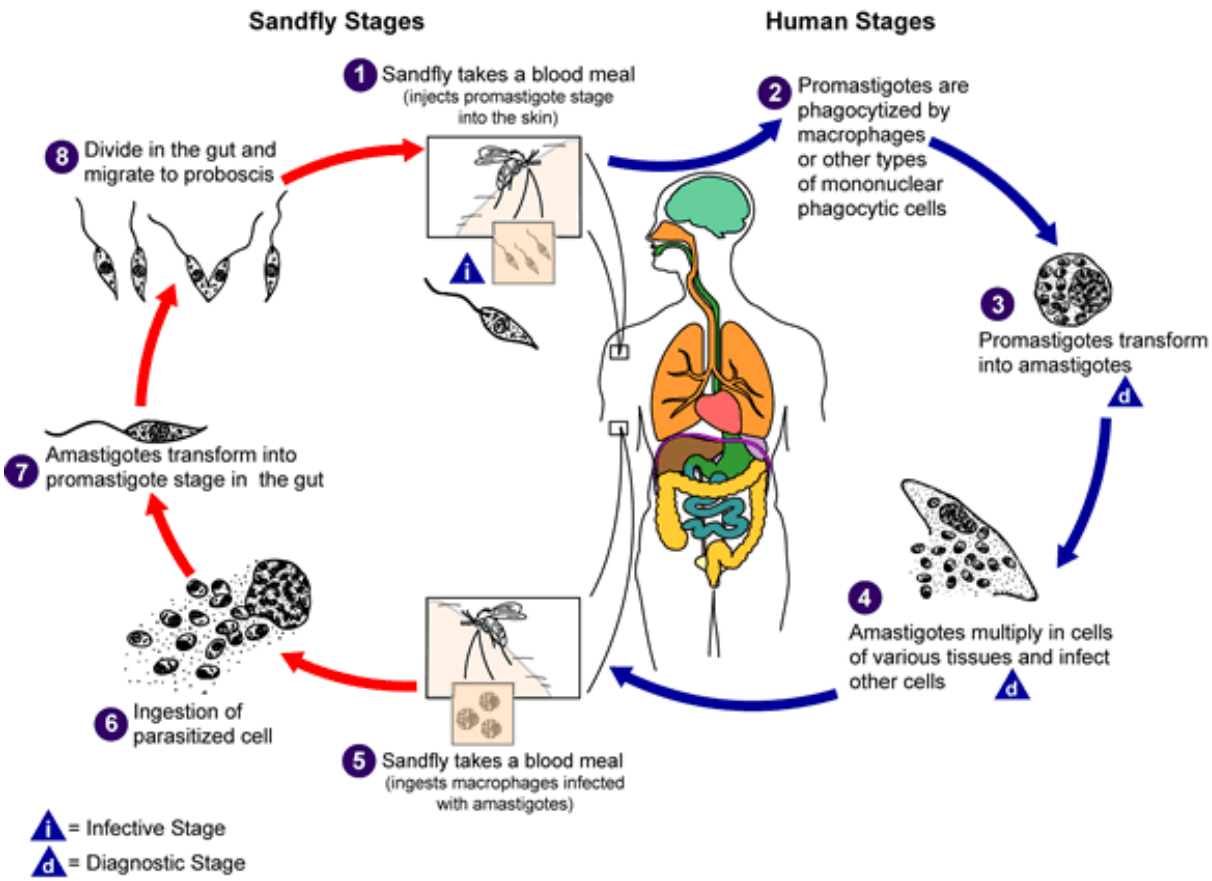

Figure 1.3: Lifecycle of the Leishmania parasite

Infected sand flies inject *Leishmania* promastigotes into the skin of a mammalian host, when taking a blood meal. Injected promastigotes are phagocytosed by macrophages, lose their flagella, and transform into amastigotes. Proliferation of amastigotes then takes place inside host cells, with dissemination to other sites such as the liver, spleen and bone marrow depending on the species of *Leishmania* and host factors. Sand flies themselves become infected after ingesting a blood meal, with the amastigotes undergoing a series of developmental changes within the sand fly to become promastigotes. Adapted from the Centers for Disease Control (CDC 2023).

1.1.2 Healthcare burden and clinical manifestations

The World Health Organisation (WHO) has defined the leishmaniases as a neglected tropical disease, for which new treatments and therapies are desperately needed (Wilson et al., 2020). It is a complex disease for which no human vaccine has reached market. Leishmaniasis is present in close to 100 countries and over a 100 million people worldwide are at risk of developing the disease every year (Alemayehu et al., 2020). 1 billion people reside in endemic regions, leading to an estimated 1 million new cases per annum (Saleh, Fazlarabbi Khan and Rowshan Kabir, 2019; Melkamu, Beyene and Zegeye, 2020; Kevric, Cappel and Keeling; Alemayehu et al., 2020). The mortality associated with this disease burden is significant with up to 20,000 deaths annually (Das et al., 2016).

The clinical spectrum of disease in those afflicted by leishmaniasis is broad. This is determined by the species of infecting *Leishmania* parasite as well as host-parasite interactions, with distinct geographical regions associated with specific parasite, and often vector species. The clinical manifestations resulting from these infections are informally termed to reflect the geographical region of preponderance with the prefix ‘Old World’ relating to disease in the Middle East, Africa, and Asia and the term ‘New World’ relating to disease in the Americas. It is notably absent from Australasia, the Pacific Islands and the Antarctic (Kevric, Cappel and Keeling).

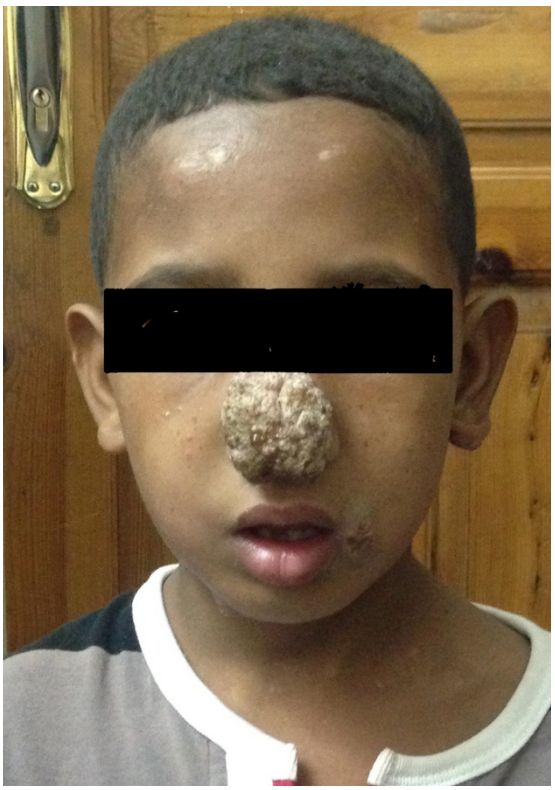

Figure 1.4: Photographic depiction of leishmaniasis infection

A Sudanese child with cutaneous leishmaniasis affecting the majority of the nose, prior to intralesional therapy. Image courtesy of Professor C Lacey, University of York.

The most commonly reported form of leishmaniasis is cutaneous leishmaniasis (CL) (Figure 1.4). Typically, this results in skin lesions that have the potential to self-heal, sometimes with ulceration. The potential for ulceration is dependent upon the infecting *Leishmania* species and also the localised response to pathogen / host derived factors. Causative species of CL include *L. major* and *L. tropica* in the Old World, and the *L. mexicana* complex and the *L. (Viannia) braziliensis* complex in the New World. The cutaneous leishmaniases cause significant morbidity and the skin changes are associated with significant stigma in those affected. The lesion forms typically at the bite site and are often seen on exposed areas including the face, with children disproportionately affected. CL caused by New World species is typically more serious and has a higher degree of morbidity and mortality. The resultant disease can affect the mucosa, so called mucosal leishmaniasis (*L. (Viannia) braziliensis*). Variations include diffuse cutaneous leishmaniasis (*L. amazonensis*) and disseminated cutaneous leishmaniasis (*L. (Viannia) guyanensis*). *Leishmania aethiopica* infection in Ethiopia is an interesting case study as the only species that is associated with the entire range of cutaneous disease (van Henten et al., 2018).

Visceral disease (VL), also known as Kala-Azar, is associated with organ involvement and other systemic sequelae and is caused by parasites of the *L. donovani* complex. Clinical features include hepatomegaly, splenomegaly, bone marrow suppression, constitutional symptoms, and an increased incidence of secondary infections (Lewis et al., 2020). Given this constellation of symptoms, it is frequently misdiagnosed as a mimic of neoplastic and inflammatory processes (Schwing et al., 2019; Santana et al., 2015). The majority of cases are reported from seven countries: Brazil, Ethiopia, India, Kenya, Somalia, South Sudan and Sudan. Bihar state in India has the highest disease burden, accounting for 70% of all cases from India (Das et al., 2016).

After treatment of VL, an appreciable number of patients may develop post-kala-azar dermal leishmaniasis (PKDL). PKDL is thought to be predominantly immunologically mediated Click or tap here to enter text.and linked to persistent parasites in the skin lesions (Zijlstra et al., 2017). PKDL lesions remain infectious to sand flies and act as important infection reservoirs which can lead to ongoing transmission complicating control efforts (Le Rutte, Zijlstra and de Vlas, 2019; Zijlstra et al., 2003; Desjeux et al., 2013). PKDL is typified by

progressive maculopapular eruptions, which can later become nodular. Furthermore, the HIV epidemic, where *Leishmania* is considered an opportunistic infection, has heightened awareness of the impact of *Leishmania* infection on immunosuppressed individuals (Albrecht et al., 1996).

1.1.3 Environmental and human impact on leishmaniasis control

The infection reservoir is complex and difficult to fully categorise, typified in the Indian subcontinent where anthroponotic transmission was seen as the most important reservoir, but recent evidence is suggestive of an important canine reservoir (Kushwaha et al., 2021). In some regions, such as the Americas, zoonosis is the sole means of transmission. Increasingly climate change has been mooted as an important consideration in leishmaniasis transmission and control. Furthermore, in endemic areas public awareness of the leishmaniases, as well as understanding of its prevention, control and treatment, is limited. Coupled with limited access to healthcare, this propagates the infection reservoir (Alemayehu et al., 2020; Melkamu, Beyene and Zegeye, 2020; Saleh, Fazlarabbi Khan and Rowshan Kabir, 2019). The burden of clinical need is therefore unmet, and an effective vaccine could potentially control the spread. There are several environmental factors that are in common with other tropical diseases. Increasing temperatures bring increased risk of famine, and the resulting malnutrition is strongly associated with increased risk of leishmaniasis acquisition in endemic regions (Anstead et al., 2001). The mechanism of this acquisition is poorly understood but is thought to be associated with an altered innate immune response, and early visceralization is thought to be the outcome (Carrillo et al., 2014). In addition, malnutrition in the context of VL is also associated with the potential for co-infection with other infectious diseases (Mengesha et al., 2014). As control of new infection events is established in geographical areas, this increases the risk of outbreaks by virtue of an increased population of infection-naïve individuals, further highlighting the potential impact of vaccine-induced immunity. Asymptomatic carriage and PKDL also contribute to the human infection reservoir.

Increasing temperatures in traditionally temperate climes may also increase suitability of regions to vector development as well as the sand fly biting rate, and therefore an increase

in exposure events (Koch et al., 2017). This has been observed with other arthropod-borne tropical infections in regions not typically associated with their spread (Cochet et al., 2022). Given the optimum temperature for parasite development in sand flies is 25˚C, a reduction in the parasite incubation period in the vector could also be observed (Chalghaf et al., 2018). It has been demonstrated that millions of individuals, particularly across Asia have been exposed chronically to arsenic in drinking water. This was associated with a large number of antimonial treatment failures and a subsequent public health crisis. Tube wells, a type of well which is characteristically tube-like and bored underground, have been installed in a shallow manner leading to much of this contamination of groundwater. This is compounded in 2 ways – by rapid depletion of groundwater such as in areas where there is population expansion and industry, but also chronic low-level arsenic exposure. Pentavalent antimonials, such as sodium stibogluconate, have been used in the treatment of leishmaniasis in these regions and are based on the chemical element antimony, closely related to arsenic. It is hypothesised that chronic low-level exposure of parasites contained within the liver and spleen of individuals, to arsenic, has thus led to cross-resistance to antimonials (Perry et al., 2011, 2015).

1.1.4 Current therapy issues

There are several treatments currently available for leishmaniasis (reviewed elsewhere: (Choi et al., 2021; Bekhit et al., 2018; Chakravarty and Sundar, 2019)), although many have a significant side effect profile, and all currently available drugs have now been associated with drug resistance (Ponte-Sucre et al., 2017; Rijal et al., 2003; Thakur et al., 2001; Choi et al., 2021; Bekhit et al., 2018; Chakravarty and Sundar, 2019). Pentavalent antimonials have been in use for decades and were one of the first major drug groups used, named for the structural inclusion of the chemical element antimony. When the first antimonial, urea stibamine, was introduced in India, it caused a significant drop in cases, although was associated with a number of side effects (Brahmachari, 1940). Newer antimonials such as sodium stibogluconate (SSG), are still poorly understood in terms of their exact mode of action, although it is thought to cause DNA fragmentation (Chakraborty and Majumder, 1988). SSG has significant local inflammatory effects including thrombosis during administration via intravenous lines or devices. It is also associated with pancreatitis,

diarrhoea and vomiting, myalgia, and rarely anaphylaxis (Parkash, Laundy and Durojaiye, 2023). It must be given as a daily parenteral injection which can limit its use. Resistance has been reported in part due to misuse on the Indian subcontinent (Rijal et al., 2003; Thakur et al., 2001). Amphotericin B, used for many decades, has been an effective anti-leishmanial drug as well as showing activity against invasive fungal disease, although has a considerable side effect profile (Maertens, 2004). This can manifest as fever, vomiting, headache, hypotension, tachypnoea and acute kidney injury, as such a newer liposomal formulation has been developed. Liposomal amphotericin B has an improved side effect profile, but is expensive and resistance has been reported (Lemke, Kiderlen and Kayser, 2005; Purkait et al., 2012; Choi et al., 2021). Newer drugs such as paromomycin, pentamidine and the oral agent miltefosine have been developed as antileishmanials. These drugs also have issues with adverse effects as well as evidence of drug resistance (Bekhit et al., 2018).

1.1.5 The vector and the parasite

Sand flies are found in temperate climates where they bite thousands of people daily. They are central to the life cycle and transmission of the *Leishmania* parasite. The life cycle of the vector itself is complex, although relatively short (see Figure 1.5). Mammalian blood meals are taken exclusively by the female sand fly primarily to facilitate egg production and oviposition, from both *Leishmania*-infected and uninfected hosts. Blood feeding behaviour has also been shown to enhance infectivity of vectors (Serafim et al., 2018). The *Leishmania* parasite has evolved over many thousands of years to become adept at surviving within the sand fly vector and within infected hosts (Akhoundi et al., 2016)(Lynn and McMaster, 2008). For example, it has been demonstrated that the *Leishmania* parasite manipulates infected hosts in order to become more attractive to female sand flies to promote further feeding, and therefore disease spread (O'Shea et al., 2002; Nevatte et al., 2017; da Rocha Silva et al., 2019; Staniek and Hamilton, 2021). These parasites have evolved to evade proteolytic activity of sand fly gut enzymes after ingestion by the sand fly following a blood meal from an infected host (Schlein and Romano, 1986). The parasite must then escape a peritrophic matrix and move towards the sand fly midgut from the hind end (Sádlová and Volf, 2009; Gossage, Rogers and Bates, 2003). In parallel to these processes, ingested amastigotes, which are intracellular, transform within the sand fly to promastigotes, which are motile. A

further series of developments and transformations then occurs to help prepare the parasite for movement proximally through the sand fly (Gossage, Rogers and Bates, 2003). The parasite also evades defecation in the bloodmeal by sand fly, by attachment to the midgut (Pimenta et al., 1994). Recent work has elucidated this attachment further and postulated a novel mechanism for target by future vaccines (Hall et al., 2020). The *Leishmania* parasite is then able to associate with promastigote secretory gel (PSG), which it influences the sand fly to produce. PSG blocks the sand fly stomodeal valve resulting in regurgitation of *Leishmania* promastigotes at the next blood meal and can also lead to increased parasite load and chronic infection at the host skin (Rogers, 2012). Sand fly saliva is also emitted at blood meals to the site of inoculum of parasite. Multiplication of amastigotes in the host then takes place within phagocytes, infecting other cells when the host cells burst (Lawyer et al., 2017). Infected sand flies have also been shown to display distinct feeding behaviour, which increases transmission and may thus increase the infection reservoir (Beach, Kiilu and Leeuwenburg, 1985).

Sand fly salivary gland proteins have also been shown to have an important local effect, by increasing parasite load and lesion size (Lestinova et al., 2017). Much of the *Leishmania* vaccine research has focused on the integral role of these proteins in initiating infection and associated long-term immunity following initial exposure. It has been demonstrated that primates exposed to sand fly salivary gland proteins, either through direct inoculation with specific salivary gland proteins or exposure to uninfected sand flies, demonstrated *Leishmania*-specific CD4(+)IFN-γ(+) immunity with a decrease in parasite load and disease manifestations (Oliveira et al., 2015). These sand fly salivary gland components have been mooted as vaccine candidates vectored with a *Leishmania* backbone (Lajevardi et al., 2022). It may be important to understand the impact of sand fly exposure on populations in attenuating immunity to further *Leishmania* challenge by testing for sand fly salivary gland antigen responses. Anaphylaxis has been reported uncommonly from some biting and hematophagous insects. However, no cases of anaphylaxis have been linked to phlebotomine sand flies in the literature. In Australasia, the term ‘sand fly’ is also used to describe many flying and biting insects that are sometimes associated with anaphylaxis and bite reactions, but not necessarily phlebotomine sand flies, which has further complicated the informal nomenclature and association with anaphylaxis (Crosby, 1973).

The presence of leishmaniavirus (LRV) may also have an impact on infectivity and disease severity. LRV are RNA viruses that infect protozoa, and in particular *Leishmania* parasites. The role of LRVs in disease is not fully understood, although they may alter the disease course and correlate with increased severity (Adaui et al., 2016; Bourreau et al., 2016; Ginouvès et al., 2021; Ives et al., 2011). LRV has been associated with several species of *Leishmania*, with potential correlation with severe New World and visceral disease, but is of uncertain significance in the context of CL (Scheffter et al., 1995).

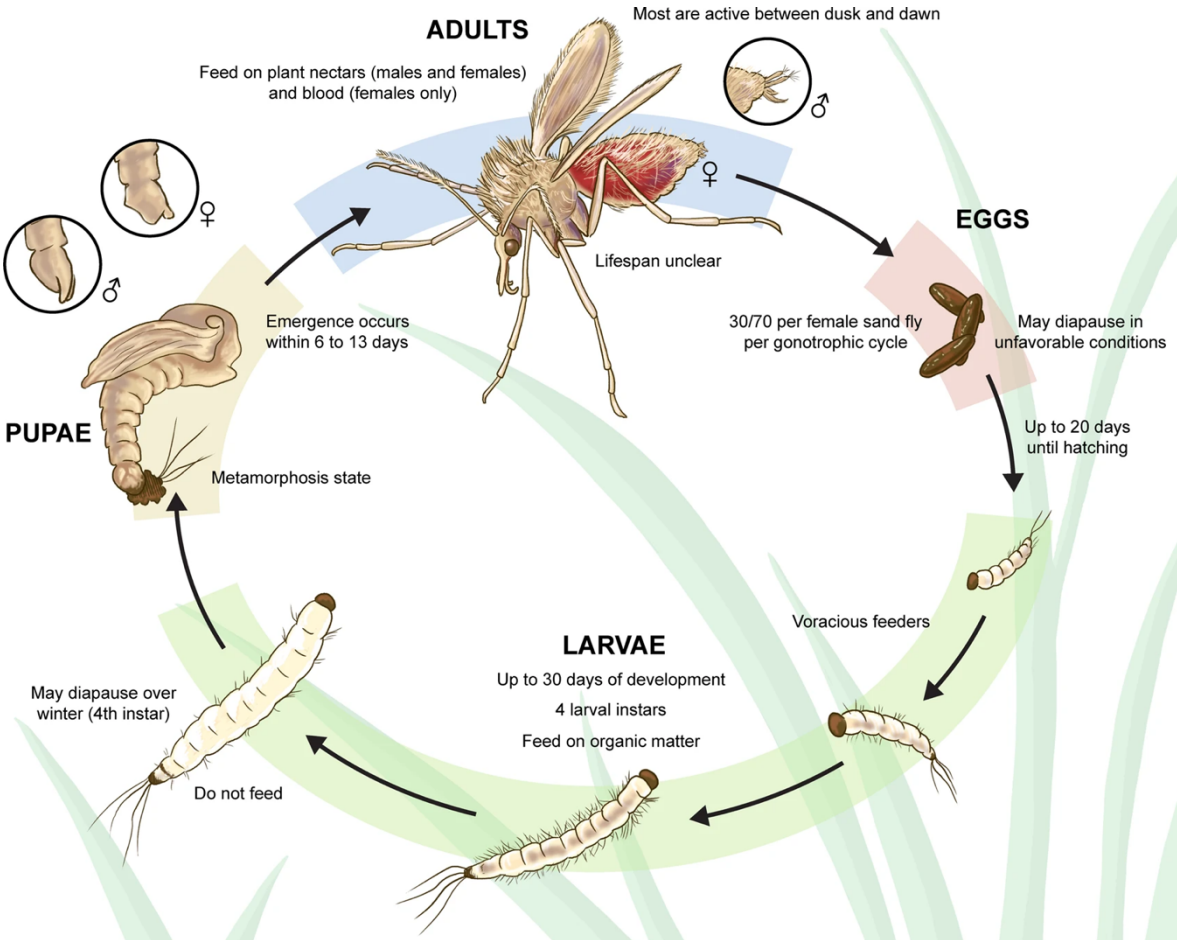

Figure 1.5: Sand fly life cycle

The sand fly life cycle is made up of 4 distinct stages, known as instars. Adapted from (Cecílio, Cordeiro-da-Silva and Oliveira, 2022).

1.2 Leishmanization

Longstanding practice in *Leishmania*-endemic countries, particularly in the Middle East, is the method of leishmanization. This practice involves inoculation of parasite/infected material, to anatomical areas that are usually covered and where scarring is not noticeable, such as the buttocks. Typically, this inoculate contained *Leishmania major*, obtained from a person with an active cutaneous lesion, using a sharp implement to derive parasite and then introduce into the recipient. The observation was made that such practice, after healing of the primary lesion, protected the recipient from further *Leishmania* infections after re-exposure throughout their lifetime (Mohebali, Nadim and Khamesipour, 2019a). It was originally thought by many observers however that lesions were the result of a localised inflammatory reaction and possible secondary infection (Marzinowsky, 1924). The immunological basis for a *Leishmania* vaccine is based on the observation that most individuals infected with *Leishmania,* do not succumb to reinfection thereby demonstrating likely robust natural immunity (Alvar et al., 2013; Nagill and Kaur, 2011). In the vast majority of cases at least partial immunity is demonstrated accompanied by an attenuated disease course. The practice of leishmanization, and the observed protective immunity has however precipitated further interest in using *Leishmania* parasites to test vaccines. The method of leishmanization has been largely discontinued due to ethical reasons, as well as the risks associated with the procedure, namely persistent lesions.

1.3 Imported infections to the UK

Autochthonous cases have not been reported in the UK, whereas imported infections, although rare, do comprise a significant case load for infectious disease units in the UK (Marks et al., 2016; Wall et al., 2012) and Europe (El Hajj et al., 2004). Cases not linked to travel have been reported rarely in non-endemic settings (Bogdan et al.), as well as increasingly the presence of the sand fly vector in temperate climates (Naucke et al., 2008). Increased use of outpatient parenteral antimicrobial therapy (OPAT) programmes has facilitated treatment of *Leishmania* infections requiring long-term parenteral treatment, in common with many other NTDs, particularly in endemic settings. OPAT has been shown to be a safe, clinically efficacious and cost-effective treatment modality (Durojaiye et al., 2018). Although cases of leishmaniasis represent a small proportion of imported infections to the

UK, the prolonged parenteral therapy required, dependent upon the species of *Leishmania* involved, represents an important healthcare resource burden. Personal communication with the UK Health Security Agency (UKHSA) has suggested that although imported infections occur frequently, data on causative species or disease manifestation is not recorded. Between 1996 to 2020, on average, 28 cases per year were reported to UKHSA. This data is highly likely to be incomplete given leishmaniasis is not currently a mandatory notifiable disease in line with many other imported infections such as malaria in the UK, and so reporting is dependent upon local hospital practices and any need for clinical confirmation with UKHSA.

I conducted a small retrospective analysis of all episodes of leishmaniasis treated at a large regional Infectious Diseases outpatient centre over a 13-year period to further determine management issues in a UK setting and any potential application to other settings (Parkash, Laundy and Durojaiye, 2023). The absolute number of cases of treatment was small – 26 episodes noted in total. All cases involved intravenous therapy for leishmaniasis, with 34.6% of cases identified as visceral leishmaniasis using serological methods (direct agglutination test). The majority (66.4%) of cases were cutaneous leishmaniasis, confirmed after tissue biopsy, and subsequent PCR speciating to at least subgenus level. 64.7% of all cutaneous disease presented with limb involvement. On average 14.2 bed days were saved per episode (SD 12.5). Current estimates suggest that inpatient treatment costs an additional £385.59 per patient per day, compared to outpatient treatment (Guest et al., 2020). This therefore represents an average estimated cost saving of £5398.26 per episode, compared to inpatient treatment. *Viannia* subgenus species were most common (42.3%) and associated with a history of travel to South America. All cases involved a compatible travel history, and no autochthonous cases were identified. The study demonstrated the use of effective and cost-effective outpatient treatment for leishmaniasis, despite the complexities in therapeutics. This has implications for the treatment of many other neglected tropical diseases that have been traditionally seen as requiring inpatient treatment in non-endemic settings. This also suggests that *Leishmania* human research is potentially feasible using an outpatient approach, which is distinct to other CHIM studies such as those involving Falciparum malaria. These outcomes also reinforced the importance of clinical history and examination to ensure naïve populations when required in clinical research.

1.4 Protective immunity to *Leishmania*

The interaction of parasite, host and vector factors is complex but crucial to the host immune response and disease course following infection. Many host cell types are infected, but the predominant cell type where promastigote transformation takes place are within the phagolysosomes of macrophages and other mononuclear phagocytic cells. In these cells, promastigotes are transformed into amastigotes, where they replicate intracellularly. Early immune responses are also determined at the introduction of parasite at the skin, by the sand fly salivary gland proteins also introduced in parallel.

Innate responses are mediated at the level of the skin predominantly by macrophages, but also include dendritic cells and neutrophils where phagocytosis may take place. The initial response involves neutrophil recruitment which mediate parasite killing, an effect which can be moderated by the presence of salivary gland elements. Conversely neutrophil interaction with phagocytes can result in macrophage activation and protection of the contained parasites. Pro-inflammatory cytokine production then takes place after activation of phagocytes by *Leishmania* cell surface molecules. As *Leishmania* is an obligate intracellular parasite, complement activation is important in facilitating parasite clearance.

Rapid reduction in parasitaemia and local parasite load occurs after complement activation and lysis of promastigotes. *L. major* is particularly susceptible to this innate mechanism of killing (Gurung and Kanneganti, 2015). The classical, lectin and alternative pathway are all thought to be involved and result in generation of C3b, resulting in deposition of the membrane attack complex and subsequent lysis and phagocytosis of the *Leishmania* parasite. The alternate pathway is deemed to be the most important pathway at amastigote killing during this mechanism. The classical pathway is also important and acts much more rapidly than the alternative pathway as clearance of promastigotes parasite is not dependent solely on the production of the membrane attack complex. The evidence for the lectin pathway is limited, but *L. major*-lectin binding has been shown to take place (Gurung and Kanneganti, 2015).

At the dermis recruitment of neutrophils occurs following sand fly biting, mainly due to IL-8. Phagocytosis by neutrophils is facilitated by C3b following activation of the complement cascade. Further phagocytosis of these *Leishmania* containing neutrophils occurs by macrophages, after they release Macrophage Inflammatory Proteins (MIP) (Scott and Novais, 2016). MIP is also involved in monocyte recruitment, which produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) resulting in further parasite killing (Romano et al., 2017).

The role of macrophages is described in both parasite replication and elimination. The *Leishmania* parasite has evolved to develop mechanisms of macrophage polarization in response to host factors which has allowed diverse roles in infection and subsequent diverse disease outcomes. The subtypes of macrophages that may develop are the proinflammatory M1 (classically activated macrophages) and the repair and anti-inflammatory associated M2 (alternatively activated macrophages). The M1 phenotype is mediated predominantly by Th-1 cells, characterized by the production of IFN-γ, resulting in ROS and NOS production by macrophages. The *Leishmania* parasite has evolved protective mechanisms to abrogate these mechanisms by interfering with cell signalling and nuclear physiology by use of Lipophosphoglycan (LPG) and leishmanial metalloprotease gp63. Parasite survival in contrast is facilitated by the M2 phenotype, activated by Th-2 cytokines. The M2 subtype produce Arginase-I, IL-10 and TGF-β, which leads to reduced NOS production and therefore parasite sparing. These macrophages are involved in hosting *Leishmania* parasite, which modulate the macrophage as a site for promastigote transformation into amastigotes with subsequent replication and cell rupture, further propagating infection.

Other cell types of importance include dendritic cells and natural killer cells. Dendritic cells serve as host cells during infection and can also have a variable role in infection depending on presence of co-stimulatory molecules. This effect can vary depending on *Leishmania* species. Natural killer cells generally have a protective role and their presence promotes healing particularly in CL (Pacheco-Fernandez et al., 2021).

T-cell mediated immunity has been well characterised, although the balance between T-cell mediated immunity and pathology with respect to leishmaniasis is complex. The T-cell (CD4/8+) response is the main mediator of cytokine expression, adaptive immunity and therefore immune control in *Leishmania* infection. CD4+ Th1 cells induce phagocyte activation by IFN-γ and TNF-α production. On the other end of the spectrum a Th-2 mediated response is associated with IL-4, IL-10 and IL-13 production, and the resultant effect is protective, particularly in CL. IL-10 is however particularly notable in VL where it is associated with worse outcomes. CD8+ cells target phagocytes containing parasites and modulate clearance, although can have the opposite effect with some species. Memory T-cells modulate subsequent re-infection following a primary infective insult and demonstrate durable immunity in VL, although their role in CL is not well understood (Pacheco-Fernandez et al., 2021; Carneiro et al., 2020; Varikuti et al., 2018).

From what is known about B cell-mediated immunity in Leishmania infection, this has a limited role. B-cell activation in response to Leishmania parasite in the host tissues results in clonal expansion, however there is little evidence for their role in antibody mediated intracellular parasite killing. However, antibodies are produced in sufficient quantities to often allow for serological diagnosis, particularly with respect to visceral disease (Pacheco-Fernandez et al., 2021).

It is also important to consider the effective immune response induced by sand fly salivary gland protein on the host (Laurent et al., 2013; Lestinova et al., 2017; Oliveira et al., 2015; Aronson et al., 2021). Although it is known that salivary gland proteins enhance infectivity when co-delivered with *Leishmania* parasite (Ockenfels, Michael and McDowell, 2014a), there is a protective effect against later parasite exposure, which is largely mediated through delayed-type hypersensitivity (Belkaid et al., 2000; Oliveira et al., 2013). This immune phenomenon is thought to occur both locally and systemically, triggering a mainly Th-1 type response (de Araujo et al., 2024). The impact of the sand fly microbiota can both positively and negatively impact parasite survival, given bacteria are emitted following sand fly biting (Pacheco-Fernandez et al., 2021; Dey et al., 2018a).

Given the complex and numerous mechanisms of immune control in natural infection and the digenetic lifecycle of *Leishmania*, as well as the numerous clinically relevant species and strains, vaccine research has employed a number of different strategies discussed elsewhere in chapter 1. The genome is highly conserved amongst those species causing human disease and brings into focus the possibility of a pan-species vaccine (Wincker et al., 1996). In part demonstrated by the practice of Leishmanization, protective immunity against further CL or VL is gained in immunocompetent individuals following primary infection, and there is also evidence for heterologous cross-protection (Tonui and Titus, 2007; Duarte et al., 2016; Romano et al., 2015). The rationale is therefore that a either a species specific or pan-species vaccine is feasible. Further strategies have involved therapeutic vaccination with respect to PKDL, which can develop following successful initial treatment of VL, particularly in Sudan. The success of Leishmanization in comparison to vaccine development may suggest that a number of approaches would be required within one vaccine. This could include a number of parasite antigens included within the vaccine architecture. Although CL and PKDL are limited in terms of a correlated biomarker, the use of the leishmanin skin test could be used to demonstrate adequate protection in VL following vaccination. Serological evaluation will only have a limited role in determining vaccine induced immunity.

1.5 Vaccine development

Several human vaccines for *Leishmania* are currently in development (Osman et al., 2017; Duthie et al., 2017; Laurent et al., 2013; Zabala-Peñafiel et al., 2020; Zhang et al., 2020), driven by the hypothesis that leishmaniasis is vaccine preventable (WHO 2020). The longstanding practice of leishmanization supports this notion, with the observation that healed CL lesions equate to durable immunity (Khamesipour et al., 2005a). As of yet no human vaccines have been licensed for use in the clinical setting (Volpedo et al., 2021). A successful vaccine could significantly alter transmission dynamics, through its impact on disease burden and transmission whilst likely allowing for savings in healthcare-related spending in endemic regions (Le Rutte et al., 2020; Lee et al., 2012). Leishmanization was considered an effective vaccination strategy, although safety concerns have limited its use.

Contemporary vaccine development has yielded several generations of *Leishmania* vaccines that have undergone clinical trials. First generation vaccines initially reached clinical trials in Brazil. These vaccines were based on whole inactivated/killed parasite with and without adjuvants, although ultimately, they were not proven to offer durable immunity despite their cost-effectiveness (Noazin et al., 2009). Second generation *Leishmania* vaccines used adjuvanted recombinant proteins (Duthie et al., 2012)(Coutinho De Oliveira, Duthie and Alves Pereira, 2020). Given their recombinant nature these second-generation vaccines have the advantage of allowing for scalable manufacture. These vaccines have shown some efficacy in animal models of CL and MCL, and safety and efficacy in a Phase 1 study inducing antigen-specific immune responses, although no further studies have been undertaken (Coler et al., 2015). The evolution to third-generation DNA vaccines has been advantageous given their safety profile and ease of manufacture, whilst allowing for ease of storage owing to their stability. In contrast to other types of novel vaccines, there have been few DNA vaccines for infectious diseases, owing in part to limitations in immunogenicity. Several third generation Leishmania vaccines have been developed, although to-date only 1 has entered human clinical trials. ChAd63-KH, has reached Phase 2 clinical trials for use as a therapeutic PKDL vaccine (Younis et al., 2021), although a further live attenuated vaccine has shown promising pre-clinical results (Zhang et al., 2020). The adenovirus vectored ChAd63-KH has shown promise, inducing a strong cellular response (Younis et al., 2021).

1.6 Controlled human infection

1.6.1 Background and historical context

Given the challenges faced in *Leishmania* vaccine research, a method is needed to evaluate any new vaccines efficiently and cost-effectively, whilst ensuring a robust approach. For many other vector-borne diseases, controlled human challenge has been used or mooted as an appropriate mechanism for measuring the effectiveness of vaccines (Darton et al., 2015). This approach is also now proposed for leishmaniasis. An effective, reproducible human challenge with *Leishmania* will therefore be invaluable in testing therapies and vaccines in future. Controlled human infection model (CHIM) studies utilise deliberate methods of human exposure to pathogens. Such studies involve deliberate infection of healthy participants in order to study efficacy of candidate vaccines, disease progression, and

human immunopathology. This can more closely replicate the natural infection cycle compared to animal studies or traditional trials. They can also give an insight into pathogenesis at predictable and early timepoints compared to observational studies. In arthropod-borne diseases such as leishmaniases, vector-specific factors are clearly important for establishing infection. The development of CHIMs for such diseases should make consideration of these factors. An important question however for this CHIM as well as future similar models, is the question of extrapolation of vaccine efficacy derived from these models. This is a challenging question and will not be answered until retrospective analysis following widespread testing of candidate vaccines.

It has been shown that the several species of *Leishmania* worldwide are closely related and have a highly conserved genome. This means in practical terms that vaccines developed against one type of *Leishmania* may elicit cross-immunity against other forms, both cutaneous and visceral as well as the several species across the globe. In terms of a human challenge, this close relation of the various species means that a study can be designed to mitigate any harm to participants by choosing a species which is easily treatable and locally contained. With respect to this, *Leishmania major* is a strong candidate for such a CHIM study. This species largely causes benign skin lesions, which often heal spontaneously. Additionally, clinical isolates of this species can be obtained from patients who have an uncomplicated disease course and analysed to document any intra-species variation.

Research concerning many infectious diseases has had meaningful outcomes as result of CHIM studies. This includes testing of new vaccines, novel discoveries from CHIMs and more accurate translation of results as compared to animal studies (Roestenberg et al., 2018),(Payne et al., 2017),(Gould et al., 2017),(Newman et al., 2015),(Kirkpatrick et al., 2016),(Ferreira et al., 2013),(Langenberg et al., 2020),(Eyal, Lipsitch and Smith, 2020),(Shah et al., 2020). There are many case studies where such models, although not resulting in successful vaccines, have improved understanding of the disease process when compared with previous animal studies. Studies involving dengue virus led to new insights into transformation of disease to dengue haemorrhagic fever. Serial blood tests including peripheral blood mononuclear cells (PBMCs), sera and plasma were also obtained which could provide a new pathway for further testing and identification of correlates of

protection and other downstream immunology (Kirkpatrick et al., 2016). In a CHIM for *Streptococcus pneumoniae,* carriage of pneumococcus was shown to be functionally significant and protective against future carriage, which could only be obtained from human rather than pre-clinical studies. Further characterization of antibody responses compared with inoculated dose was also possible (Ferreira et al., 2013). In a norovirus CHIM, the dynamics of the infection reservoir within the host were elucidated, namely absence of viraemia (Newman et al., 2015). An archived biobank from this early norovirus CHIM has also allowed a later study to determine novel diagnostics and assays (Kirby et al., 2020). In a CHIM for schistosomiasis, although this could not reproduce the impact of repeated infection in endemic settings, the utility of currently available early diagnostic tests was confirmed. Schistosoma anti-adult worm IgM is used in return travellers already, although the findings explicitly demonstrated their utility, and determined the earliest point for its use (Langenberg et al., 2020). Outcomes from a CHIM study for malaria suggested that changes in the existing vaccination approach could be more successful. In this CHIM it was observed that after administration of an adenovirus-vectored vaccine, strong cellular immune responses did not impact on parasite growth rates. The implication was therefore that vaccines inducing T-cells were unlikely to be successful and future vaccines should be directed towards achieving protective antibody titres (Sheehy et al., 2012). Where there is an absence of suitable or validated animal models, CHIM studies have been particularly beneficial. A prominent example is when considering a dengue virus CHIM. It has been shown that non-human primates do not display clinical signs of disease despite a measurable viraemia, which is distinct from the clinical picture in humans (Kirkpatrick et al., 2016; Thomas, 2013). CHIM studies are also being considered for many diseases where the prevailing scientific understanding was that controlled human infection was not feasible due to ethical and clinical concerns, including factors such as the severity of human infection and the potential for uncontrolled disease. One such example is experimental infection with bacillus Calmette-Guérin (BCG), used as a surrogate to test treatments for *Mycobacterium tuberculosis* (Carter et al., 2023).

1.6.2 History of experimental *Leishmania* infection

Leishmaniasis is widely accepted as being a vaccine-preventable neglected tropical disease, although no vaccine has yet been licenced for human use, the details of which are discussed elsewhere in this chapter (WHO 2020). Although the spectrum of disease caused by distinct species of *Leishmania* parasite is diverse, the disparate species are uniquely related, compared to many other parasitic diseases (Lynn and McMaster, 2008). It has been shown that the genomes of *Leishmania* species are highly conserved(Peacock et al., 2007),(Wincker et al., 1996), sharing some common features also with other trypanosomes (Dolezel, 1997). These findings highlight the possibility of pan-*Leishmania* interventions (Coler and Reed, 2005),(Alzahrani et al., 2017),(Cecílio et al., 2020a) and also the distant possibility of pan-trypanosome interventions (Field et al., 2017). Indeed, in mouse models it has been demonstrated that after initial intra-dermal challenge with a *Leishmania* species causing exclusively cutaneous disease, protection is gained against further challenge from a species causing visceral disease (Romano et al., 2015). These findings have precipitated ongoing research into a *Leishmania* vaccine and enhanced the potential predictive ability of any CHIM using species that cause less invasive disease.

There has also been much discussion of the use of sand fly salivary gland proteins within vaccines. Sand fly species have evolved a unique relationship with specific species of *Leishmania* and both have specific geographical distribution. This has implications for effectiveness of vaccines, with a recognition that vaccines employing proteins from specific sand fly species having the possibility of effectiveness in limited geographical areas only. Recent developments have suggested the utility of a conserved sand fly protein in contributing to a vector-based pan-*Leishmania* vaccine(Cecílio et al., 2020a),(Nacif-Pimenta et al., 2020). Indeed the presence of sand fly saliva is associated with establishment of infection, and contributes to disease manifestations. (Belkaid et al., 1998),(Oliveira et al., 2013),(Kamhawi et al., 2000a),(Ockenfels, Michael and McDowell, 2014b),(Rogers et al., 2009) Additionally it has been shown that vaccines may demonstrate degrees of protection in mouse models using needle challenge that is not seen after infection transmitted by the natural vector.(Peters et al., 2012b) The sand fly is integral to the model given the close relationship between parasite and vector in conferring infection, and indeed it has been

demonstrated (in murine models) that uninfected sand fly bite alone is protective against cutaneous leishmaniasis(Kamhawi et al., 2000a),(Peters et al., 2009). The microbiome of the sand fly may have an important effect on the behaviour of the parasite and subsequent infection establishment, with phlebotomine sand flies demonstrating an extremely diverse microbiota in comparison to other arthropods.(Jiménez-Cortés et al., 2018),(Finney, Kamhawi and Wasmuth, 2015) As such, using sand flies to confer experimental infection closely mirroring the natural infection cycle is important in ensuring the validity of results.

Numerous animal models exist for the study of most forms of leishmaniasis, which have added novel insights into the behaviour of host, pathogen and vector (Loría-Cervera and Andrade-Narváez, 2014),(Garg and Dube, 2006). However, the predictive power of these animal models in terms of human response may not be reliable (Raman et al., 2012). The two main groups of mice in animal leishmaniasis models, the C57BL/6 and BALB/c, have distinct immunological profiles in response to *Leishmania* infection and therefore translation to clinical practice may be difficult to predict (Juliana Mcelrath et al., 1987; Scott and Novais, 2016). An infected sand fly injects promastigote parasites into the dermis during feeding, and it is estimated that several hundred parasites are introduced at this time (Kimblin et al., 2008). It is difficult to replicate this using intra-dermal or other parenteral injection of parasite as used in animal models. Furthermore, vaccine development has been limited by incomplete understanding of the pathogenesis of the disease, and the complex immune response to pathogen, which can not only provide robust long-lasting immunity but also cause exaggerated acute and sub-acute responses (Kedzierski, 2010). Vaccine development is expensive and balanced against a backdrop of limited funding available in leishmaniasis research, cost-effectiveness is a necessity of vaccine development (Le Rutte et al., 2020; Gouglas et al., 2018).

1.7 Histological and immunohistochemical findings in cutaneous Leishmaniasis lesions

Leishmaniasis has a number of clinical presentations and can mimic several other disease processes. The differential for a lesion that appears to be cutaneous leishmaniasis lesion is therefore wide and encompasses both infective and non-infective aetiologies (Handler et

al., 2015). This could be complicated by the presence of parallel disease processes such as secondary bacterial infections which are thought to be present in at least 20% of clinically presenting CL (Meireles et al., 2017). In addition, treatment can be complex and be associated with many side effects as demonstrated in a small study I conducted and described elsewhere in chapter 1 (Parkash, Laundy and Durojaiye, 2023). It is therefore important to establish the key histological and immunohistochemical findings that can aid in differential diagnosis for a cutaneous Leishmaniasis lesion, in addition to known PCR, serological, parasite culture and other methods. The diagnosis hinges on identifying the presence of *Leishmania* amastigotes in clinical samples by either direct microscopy or molecular methods (Abadías-Granado et al., 2021).

The main dermoscopic feature of CL lesion includes erythema, which is of course very common in many other conditions (Taheri et al., 2013). Other key dermoscopic findings include 'starburst'-like patterns, central erosion and ulceration, tear-drop structures and hyperkeratosis. In addition, an increase in vascularisation in a polymorphic pattern may be noted (Llambrich et al., 2009; Taheri et al., 2013; Yücel et al., 2013). This is more common when parasite load is higher (Salman et al.).

The use of histology to positively identify a CL lesion is beneficial as part of a combined approach in diagnosis, although one study suggests histopathological confirmation after initial clinical diagnosis was only successful in 59.6% (34/57) of cases (Al-hucheimi, Sultan and Al-Dhalimi, 2009). The usual method of obtaining a sample is by punch biopsy or excisional/incisional biopsy, ideally with healthy tissue included (Handler et al., 2015).

Early lesions usually present as nodules, papules, ulcers or crusted plaques, with common macroscopic appearances between Old World and New World CL. In early disease, an inflammatory predominantly dermal infiltrate composed mainly of macrophages may be present. This inflammatory change is diffuse but dense. The findings at this early stage include an abundance of *Leishmania* amastigotes, usually within macrophages and found beneath the epidermis. The amastigotes are small and round, and are 2 to 4 μm in size, with a characteristic kinetoplast. Langerhans cells which migrate to the dermis can also contain amastigotes. If the parasite load is high, then amastigotes may be seen extracellularly. The

inflammatory infiltrate may spare an area of the papillary dermis also known as the Grenz zone. This inflammatory reaction then results in aggregation of lymphocytes, plasma cells eosinophils and polymorphonuclear cells, but are fewer in number to macrophages in the dermis. The dermal infiltrate can lead to destruction of specialised cell groups (appendages), such as sebaceous and sweat glands, and hair follicles which is most visible as atrophic scarring. Epithelioid cell granulomas with surrounding lymphocytes can also be present. Discrete necrotic regions may be found as the lesion progresses. More chronic lesions demonstrate a larger number of plasma cells, and granulomas can occasionally form (Mehregan, Mehregan and Mehregan, 1999; Waduge, 2010; Singh and Ramesh; Al-hucheimi, Sultan and Al-Dhalimi, 2009; Abadías-Granado et al., 2021; Meireles et al., 2017; Handler et al., 2015). Compared to other species, *Leishmania major* associated CL lesions have a tendency for fewer granulomas, and fewer lymphocytes associated with the inflammatory infiltrate, but a higher number of plasma cells and polymorphonuclear cells (Boussoffara et al., 2019). It is hypothesized that this leads to better parasite survival rates in *L. major* as a result of limited macrophage activation following uptake of apoptotic neutrophils(Scott and Novais, 2016). Immunohistochemical staining, typically with haematoxylin and eosin, demonstrates an abundance of CD4+ T cells compared to CD8+ T cells in *L. major* , with variable CD3+ T cells and an absence of natural killer cells. In general, *L. major* lesions have lower expression of CD4+ and CD8+ compared to other species (Boussoffara et al., 2019; Wijesinghe et al., 2022; Gaafar et al., 1999). Cytokine expression in CL lesions is also distinctive and in keeping with a Th-1 type response with IFN-γ and IL-10 predominating. IFN-γ is important in immune control, and an inadequate Th-1 response is associated with increase *L.major* susceptibility. IL-10 number decrease as lesions become more chronic. *L. major* in particularly has shown to be associated with a higher expression of IL-8.

1.8 History of *Leishmania* CHIMs

Leishmanization practice was largely abandoned due to reports of localised reactions, including significantly prolonged local lesions, in comparison to the recognized disease progression following infection with *Leishmania major* (Khamesipour et al., 2012), although the practice of leishmanization still took place until recent times in Uzbekistan (McCall et al., 2013). The 20th century heralded a series of studies which improved the understanding of

leishmaniasis disease, the parasite, transmission and additional human implications. Early work demonstrated human infection after inoculation of parasite from canines, which illustrated the potential for *Leishmania* species to infect and manifest similar disease across species (Adler and Theodor, 1930). This was one of the first suggestions of an infection reservoir across species that had relevance to clinical outcomes. The first documented experimental transmission to humans was in 1907, using subcutaneous inoculation (Marzinowsky, 1924). The practice of Leishmanization has been studied more recently to categorize its reproducibility and utility to study new vaccines and even for use as a possible live vaccine (Berberian, 1939; Senekji and Beattie, 1941; Adler, 1940; Khamesipour et al., 2005a; Melby, 1991). This was facilitated by improved parasite culture techniques (Senekji, 1939) and improved understanding of good manufacturing practices (GMP). *L. major* is the most appropriate species to use in a leishmanization model given its more benign disease course compared to other species infection humans. However, the risk of introduction of *L. major* to non-endemic settings via this method is a consideration. This is particularly important given the observation that altered sand fly feeding behaviour as a result of infection can rapidly increase the spread of *Leishmania* and impact on the infection reservoir (Beach, Kiilu and Leeuwenburg, 1985).

The association of the *Leishmania* parasite with the sand fly was suggested after work in the early 20th century (Adler and Theodor, 1925a). The transmission of *Leishmania* using the sand fly as a potential vector, was then confirmed in experimental human infection. These experiments did not directly involve sand fly exposure to humans however (De La Tribonniere, 2000). Experiments involving *Leishmania*-infected sandflies, have for the most part involved animal models. Xenodiagnosis is a method using examination of the transmission vector to assess for presence of disease. This method was then used to confirm transmission from infected humans to non-infected sand flies, in the first documented experimental human exposure to sand flies (Adler and Theodor, 1927b). Further original work by Adler and Theodor confirmed the transmission of *Leishmania* from phlebotomine sand flies directly to humans. These results have since been confirmed and replicated many times, along with experimental human infections using parenteral methods (Lainson and Strangways-Dixon, 1963; Swaminath, Shortt and Anderson, 2006; Adler and Ber, 1941; Melby, 1991; Adler and Theodor, 1925b, 1926a, 1926b). This body of work revealed the life

cycle of the *Leishmania* parasite in the sand fly and led to the finding of transmission via sand fly bite. It was also noted that artificial sand fly feeding on infected mammalian tissue was comparable to transmission from human to sand fly (Adler and Theodor, 1927a, 1929). Subsequent studies replicated transmission using many different sand fly and *Leishmania* species (Strangways-Dixon and Lainson, 1966; Guirges, 1971; Naggan, Gunders and Michaeli, 1972). Given the complex relationship between pathogen, host and vector, several studies sought to determine the nature of the sand fly bite reaction on humans, including in the absence of parasite. Several such studies have taken place over the last hundred years, with continual evolution in understanding of the importance of the sand fly bite in initiating and establishing disease (Theodor, 1935; Oliveira et al., 2013). The importance of sand fly salivary gland protein as an independent factor has also been strengthened with the demonstration of protection against further challenge with *Leishmania* in murine models (Kamhawi et al., 2000a; Belkaid et al., 1998).

Contemporary studies of experimental human infection have examined the immunological basis and consequences of infection. One study attempted to determine cross-protection using human exposure to *Leishmania*. One human participant was exposed to *L. arabica*-infected *P. papatasi* sand flies and 3 participants received needle challenge (Peters et al., 1990). The participant exposed to infected sand flies did not develop lesions, although all participants were subsequently challenged with *L. major* and developed lesions by day 250 post-challenge. A further study, using an *L. major* stabilate made to GMP conditions, demonstrated lesion development, via needle challenge, in 19 out of 23 healthy *Leishmania*-naïve participants (Khamesipour et al., 2005a). By day 60 following challenge, the majority (74%) developed ulcerated lesions, although all lesions remained less than 3cm, and healed successfully with treatment. A proportion of the volunteers were re-challenged, although the viability of the inoculum was deemed poor. The study further reinforced the importance of sand fly salivary gland proteins for development of infection.

There is no evidence for reactivation of *Leishmania major* in Leishmanized individuals (Romano et al., 2015) and the disease course is thought to be benign in the majority of individuals. Given the potential fragility of the use of sand flies in a human experimental model, a GMP-produced *Leishmania* strain would be advantageous for use in any

subsequent needle challenge. For such a needle challenge, there would however be a question about the potential inoculation of culture media and difficulties in providing an accurate dosing of parasite.

The development of an effective CHIM for cutaneous leishmaniasis will significantly improve the timeline for production of new vaccines, as well as testing vaccines already in the pipeline at an early stage. In parallel with other disease areas, an effective CHIM is also likely to significantly improve understanding of human pathogenesis. An effective vaccine would significantly improve disease control efforts, including impacts on morbidity and mortality (Le Rutte et al., 2020). CHIM studies are however not usually a substitute for efficacy trials, and are used therefore to inform future large-scale vaccine studies. Some exceptions exist however, with the cholera vaccine, Vaxchora, gaining licensure based on data from a vaccine-CHIM trial (Chen et al., 2016; Ramanathan et al., 2019).

1.8.1.1 Ethical considerations

Deliberate human infection has been employed as a useful technique in testing hypothesised therapeutic tools for over 200 years. Carried out within an appropriate ethical framework, human challenge has been shown to be extremely effective. Over the last few decades controlled human challenges have been fine-tuned to ensure both safety and reliability of results for a wide range of infectious diseases. Deliberate infection of volunteers needs careful forethought to navigate the many ethical and safety concerns and in particular informed consent and a careful evaluation of both harms and perceived benefits. There is also significant breadth of understanding of CHIM studies by the public in LMICs (Vaz et al., 2020), and significant ethical issues to overcome to undertake a CHIM in endemic settings (Jamrozik and Selgelid, 2020b, 2020a, 2021; Njue et al., 2018a).

There has been much ethical discussion of these studies given the deliberate nature of such infections. The Hippocratic oath has been quoted as a barrier to such studies particularly with regard to a chequered history of deliberate infection in the early part of the 20th century with both Nazi experimentation and the Tuskegee syphilis studies widely mentioned within this argument. Some of the very first deliberate human challenge infections involved

smallpox, initially variolation and latterly and more famously Edward Jenner's work involving cowpox in the development of a vaccine against smallpox. A similar approach has been employed for centuries with cutaneous leishmaniasis, using leishmanization as described above.

In the last 60 years, a more structured, evidenced-based and ethical approach to deliberate infection has been employed. Scientists have sought to investigate diseases where a deliberate infection would be beneficial in either better understanding a disease or to test potential therapies (Darton et al., 2015), including diseases as diverse as influenza(Gould et al., 2017), norovirus(Newman et al., 2015), malaria(Payne et al., 2017) and dengue(Kirkpatrick et al., 2016). Although there have been no reported cases within the medical literature of anaphylaxis to phlebotomine sand fly bites, given the possible association with hypersensitivity (Oliveira et al., 2013; Belkaid et al., 2000), and the association between anaphylaxis and biting insects (Hoffman, 1987) it would be imperative to ensure safety by ensuring that provisions are in place for treatment.
Click or tap here to enter text.
It is feasible that following any CHIM study, there is a potential for retention of parasites at the site of inoculation, and potentially even satellite sites. This is particularly relevant for species of *Leishmania* that are susceptible to reactivation in immunosuppressed individuals, in particular New World species and species causing visceral leishmaniasis(de Souza et al., 2017; Postorino et al., 2011). Following treatment or biopsy of lesions, it is feasible that any remaining parasites may be susceptible to reactivation if the CHIM participant later develops a condition, or is required to take treatment, that causes immunocompromise. There are no descriptions in the literature that describe this occurrence with *L. major* following successful treatment. This may therefore prove prohibitive for use of other species as challenge agents. Parasite retention can also be beneficial in stimulating the immune response and preventing reinfection in immunocompetent individuals. There is therefore potential for CHIM participants to gain benefit from this in preventing re-infection if travelling to a leishmaniasis endemic region and if subsequently exposed naturally. However, given the importance of host-specific factors in establishing leishmaniasis infection, and manifestation of disease partly dependent on host genetics and epigenetics,

differences in host susceptibility may impact on success of any CHIM (Sakthianandeswaren, Foote and Handman, 2009; Kaye and Scott, 2011).

1.9 Conclusion and aims

Leishmaniasis is a disease with a wide spectrum of clinical outcomes, disproportionately affecting those who suffer from poverty and malnutrition. The same species has the potential to cause distinct clinical manifestations, partly determined by complex parasite-host-vector interactions. However, species are usually associated with one disease type.

Control and treatment efforts have had varying degrees of success. The COVID-19 global pandemic is likely to have far-reaching consequences for research funding for neglected tropical diseases as well as disease control in the coming years. Modelling has demonstrated that delays in the visceral leishmaniasis elimination target in India are probable, and the number of new cases are likely to rise (Le Rutte et al., 2021).

The hypothesis of the work described here is that a novel sand fly-initiated *Leishmania* CHIM is feasible, and will be demonstrate efficacy and safety. In chapter 2 I will describe public involvement work, that underpins the development of this CHIM, to evaluate public support of this work, and the potential impact on the proposed study design. In chapter 3 I describe the development of a protocol for a *Leishmania*-CHIM initially using uninfected sand flies on human participants, examining safety and efficacy. In chapter 4 I describe a CHIM clinical study using *Leishmania major*-infected sand flies, with the clinical isolate from a novel parasite bank designed for human studies. Figure 1.6 describes the enabling studies on the pathway to a *Leishmania* CHIM.

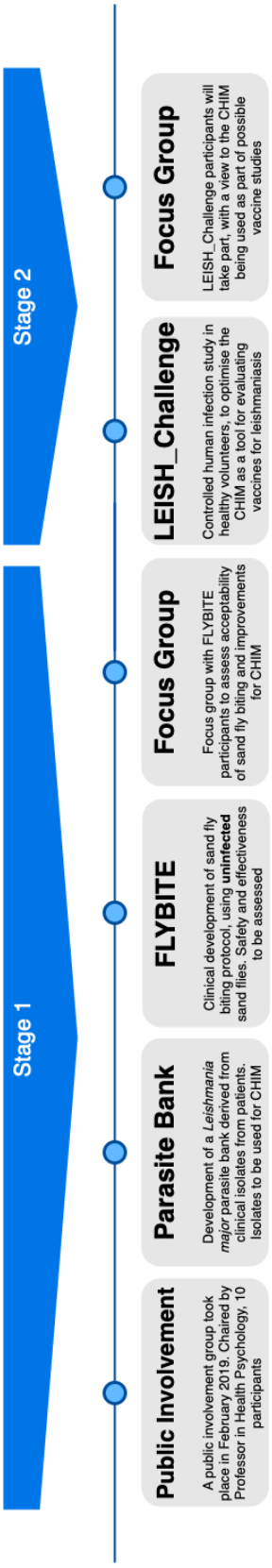

Figure 1.6: Schematic of the development pathway for the *Leishmania* CHIM

Reproduced from (Parkash et al., 2021b)

2 Chapter 2: Public perception of controlled human infection for leishmaniasis

2.1 Patient-centred healthcare and research

The principles of patient-centred care have strengthened in the UK since the mid-20th century when the term was first used by Carl Rogers in relation to psychotherapy (Langberg, Dyhr and Davidsen, 2019). Some would say that Sir William Osler was the first in the modern era to describe patient-centred care when he famously stated "*Learn to study patients, not cases— individuals, not diseases*" (Bliss, 1999). The concept has progressed over the past 50 years and is now incorporated in healthcare in the UK, both in day-to-day practice and embedded within healthcare policy. Extrapolating from patient-centred healthcare, and with the notion that good quality care is dependent upon effective parallel clinical research, high-quality research is thought to be dependent on public involvement (Bagley et al., 2016).

Awareness and funding for public involvement (PI) and engagement activities within clinical research have been supported by advisory bodies such as INVOLVE, backed by the National Institute for Health Research (NIHR), established in 2016, to support PI activities. As the need for PI has been increasingly embraced, support for such activities has subsequently been integrated into all UK research bodies and funding streams (NIHR 2023). This is typified by the UK National Health Service (NHS) Health Research Authority document 'The UK Policy Framework for Health and Social Care' published to address public involvement in research including within the ethical review of studies (NHS Health Research Authority, 2017), (NIHR 2021). Impact of research is also increasingly measured by the nature and extent of public involvement and engagement activities including by The Research Excellence Framework (REF), who include PI activity in its impact assessment (REF, 2021).

A range of terms exists to describe engagement with the public, including patient-public involvement (PPI), public involvement (PI) and patient and public involvement and engagement (PPIE), sometimes used interchangeably, and sometimes used depending on the experience of those primarily involved in the activity. PI activities foster inclusive,

collaborative working practices as well as reflexivity (Crocker et al., 2018). Given this demonstrable importance of public involvement in improved research outcomes, particularly when included at preliminary stages of study development, including better recruitment, impact on study design, retention of participants, delivery of high-quality research, PI has now a become a necessity of many funding applications (Crocker et al., 2018). The inclusion of PI can therefore allow for a more pragmatic approach to the research question, and the hypothesis may therefore be more applicable to real-world applications. By fostering this relationship between the public, patients and researchers, novel areas for research can be determined, in contrast to building a research study solely with researchers (Price et al., 2018; Liabo et al., 2020; Jinks et al., 2013). In parallel to this, there is growing evidence to suggest that greater understanding of the research area can be gained by PI as well as improved public-facing materials. Improved research literacy can therefore allow for better interface with future research activities (Price et al., 2018; Wyatt et al., 2008). Additionally, in building studies in a collaborative fashion, a shared accountability can develop as well as more cost-effective research practices (Komporozos-Athanasiou, Thompson and Fotaki, 2017; Levitan et al., 2018).

Public awareness of CHIM studies is modest both in well-resourced and under-resourced settings (Vaz et al., 2020; Njue et al., 2018a; Gbesemete et al., 2020). The recent COVID-19 pandemic and associated CHIM to test SARS-CoV-2 vaccines has however improved scientific literacy and some awareness of these themes (Killingley et al., 2022; Edwards and Neuzil, 2022). Over the same timescale so-called ‘fake news’ and misinformation with respect to themes around vaccination and illness have also proliferated (van der Linden, Roozenbeek and Compton, 2020; Roozenbeek et al., 2020; Li et al., 2022). Public engagement therefore plays an important role in making complex themes accessible, simplifying complex issues, and taking control of narratives that may become distorted without the input of science-literate individuals (Većkalov et al., 2022; Farrell and Wilkinson, 2022; Adeyemi et al., 2022).

Given the acknowledged benefits of public engagement in the development of CHIM research (Gbesemete et al., 2020), in developing a proposed controlled human infection model for cutaneous leishmaniasis, it would be prudent to engage relevant stakeholders,

including the public, to discuss the themes surrounding such a CHIM. There are limited studies to assess public awareness of issues concerning leishmaniasis (Saleh, Fazlarabbi Khan and Rowshan Kabir, 2019), and no such studies at the intersection of CHIMs and leishmaniasis. I describe here how the development of a controlled human infection model for the cutaneous leishmaniasis, was shaped by public involvement. As described previously, there is inconsistency in terms of the language of public engagement activities. As there were no patients involved in this research activity and given its use by NIHR (NIHR 2021), the term 'public involvement' (PI) was used for this exercise.

2.2 Plain English Summary of underpinning public engagement activity

Plain English summaries provide the opportunity to allow a topic to be discussed in a concise and accessible fashion. As this project required engagement of participants at all stages, a plain English summary of the outline of the project served as the backbone for all public facing literature for the duration of the project. According to the National Literacy Trust, over 7 million adults in England have 'very poor' literacy skills (equivalent to inability to read bus timetables) (National Literary Trust, 2022), and so accessible material is imperative if the public is to be appropriately engaged.

The advantage of such summaries includes allowing funders to better understand the research proposal, particularly those from non-scientific backgrounds (i.e. lay representatives). Perhaps most important are the impact on stakeholders, policy makers, other health professionals and the public at large, and these summaries can contribute to strengthening links with these groups. There are increasing calls for use of lay summaries in science communication as a result (Kuehne and Olden, 2015), and several tools made available specifically for researchers to use to achieve this (Rakedzon et al., 2017). I designed a plain English summary with relevance to the public engagement activities, and which evolved over the course of the PI activity and was influenced by its outcomes:

"Our research team is designing a type of research study known as a controlled human infection model (CHIM). In CHIM studies, volunteers are exposed to infections on purpose and then studied to help understand diseases. Similar experiments, where humans are

infected deliberately, have been used for hundreds of years to help test treatments. CHIM studies have already been used more recently to help test vaccines for diseases such as malaria.

The disease leishmaniasis, a disease affecting millions each year, is spread by the bite of an infected sand fly in tropical countries. There are currently no vaccines for leishmaniasis that are available for use in humans. It is thought that by using CHIM studies, new vaccines might be tested and then approved more quickly. Scientific researchers have had many discussions about how useful CHIM studies are, especially in terms of the science behind them, the safety of volunteers and the ethics of these studies. Researchers also understand how important it is to involve the public in designing and carrying out research, especially studies involving humans, to get an independent point-of-view. We have therefore involved the public, in some parts of designing this research, in a group discussion. We also included a person who has already taken part in a different CHIM study. These discussions have had an important effect and have changed how we plan to carry out our future research studies. We also hope that this description will encourage other researchers to include the public when planning future research." (Parkash et al., 2021b)

Plain English summary reproduced from
Parkash, V. *et al.* Assessing public perception of a sand fly biting study on the pathway to a controlled human infection model for cutaneous leishmaniasis. *Res. Involv. Engagem.* **7**, 33 (2021).

2.3 Methods

2.3.1 Recruitment and setting

Favourable ethical approval for a PI consultation group was granted by the Department of Biology Ethics Committee (BEC), University of York (Ethics reference PK201812). INVOLVE guidance (INVOLVE 2020) and the NHS Health Research Authority decision tool (Health Research Authority 2020) deemed submission to NHS Research Ethics Committee unnecessary. Documents approved by the BEC included study protocol, agenda for participants, draft FLYBITE and LEISH_Challenge design and volunteer

advertisement/volunteer information leaflets, participant consent form and plain English summary (see appendix Figure 7.1 to Figure 7.8).

The recruitment plan involved local and national advertisement campaigns, which included patient advocacy groups and national CHIM networks. Local recruitment involved recruitment through the University of York email mailing lists, poster displays, local newspapers and presentations on campus. If an individual was interested, they were then invited to contact the study team. They were asked about the reasons for entering the study and then invited to participate.10 participants (7 female and 3 male) were recruited. They included staff and students from the University of York, a participant from a previous UK-based CHIM, a previous UK-based vaccine study participant, lay volunteers from the local community and a patient research ambassador from a UK-based organisation. 6 participants were educated to higher-education level (of which 5 had a degree in science-adjacent fields). 5 participants were in paid employment, and 4 were full-time students. Age and ethnicity data were not recorded as part of the study cohort, although a lack of diversity was noted. An attempt was made to recruit patients from local and national networks who had previously been treated for cutaneous leishmaniasis and wished to be contacted, however I was unable to recruit such individuals in a timely fashion.

Participants were remunerated according to INVOLVE NIHR and UK Department of Health guidance (INVOLVE 2021) (NIHR 2021). Participants' received remuneration to the value of £25 per hour of scheduled commitment, and additional travel costs where requested. Full written and verbal consent was obtained from the participants to use audio recordings, quotations, and other data for publication.

The consultation exercise took place over the course of 3 hours, with all 10 participants present, and prior to ethical review of the interventional human studies. No patients previously treated for leishmaniasis were available for recruitment to the PI consultation. There was no virtual component, and the activity took place in-person at The University of York on the 12th of February 2019.

Participants were given sample materials for the proposed project in advance in order to allow participants to fully appraise relevant documentation and consider any relevant questions that they may wish to bring to proceedings. Participants' role's in considering the project were described beforehand in email communication, and included

i) Consideration of draft study protocols to identify any key issues
ii) Consideration of the acceptability of proposed study design
iii) Consideration of ethical issues
iv) Consideration of a plain English summary of the project
v) Consideration of the proposed recruitment to the studies, including nature of the inclusion and exclusion criteria and method of recruitment

The documents provided to participants, in addition to information about the PI activity and schedule, included information about a future CHIM for CL:

- a volunteer information leaflet for potential sand fly biting study (FLYBITE) – using uninfected sand flies
- a volunteer information leaflet for a potential *Leishmania* CHIM
- a plain English summary of the potential CHIM study and initial underpinning work
- recruitment poster for volunteers for a sand fly biting study

(see appendix Figure 7.1 to Figure 7.8).

2.3.2 Study design and approach to data collection methodology

The study was a performed in accordance with widely reported literature and guidance on Patient and Public Involvement and Engagement (Kaisler, Missbach and Kaisler, 2020). An independent facilitator was appointed, Professor Georgina Jones, Leeds-Beckett University, (GJ), to chair and steer the discussion. GJ has several years' experience as a health psychologist, which includes running PI groups and PI-focused clinical research. The wider clinical team members were also present including Professor Charles Lacey (CJL) and Professor Alison Layton (AML), both University of York.

The proceedings had 3 planned phases

1. Initial summary and overview

2. Discussion of further themes, directed by independent chair, and without clinical investigators
3. Closing remarks, clinical questions, and feedback

The study was designed to give participants the maximum opportunity to voice opinions. The method used included a mixture of a partial consensus workshop approach and elements of a modified Nominal Group technique (Rankin et al., 2016; Liamputtong, 2015; Black et al., 1999). I had planned an initial summary presentation of the project to structure the discussion and then for the discussion to be held entirely between the independent facilitator and the PI consultation group participants. The other clinical investigators and I were scheduled to be present to clarify any queries of a clinical or technical nature for the first phase only, and then to return for the final phase, with the second phase due to be the longest. Given the complex and unique nature CHIM studies and the prior research experience of some of the participants, the first phase became an extended session as participants continued to discuss and ask relevant questions of clinical investigators. This was also enhanced by the prior research-participation experience of some of the participants and also by the ethos surrounding the PI groups discussions, i.e., candour, respect for everyone's opinions and flexibility in reaching the study aims. The format therefore evolved to a more extended discussion after sequentially exploring each theme, comprised of questions posed directly to investigators. The independent facilitator role then evolved to allow for expansion of relevant topics at suitable timepoints to allow for a fulsome discussion. The clinical team members then left the meeting to allow for phase 2 to begin, and a candid discussion with the independent facilitator. The schedule was not adhered to strictly to further allow for this. Phase 3 allowed for return of clinical investigators to proceedings, and any themes not fully explored were discussed at this point including the opportunity for follow-up questions. Participants were asked after the session to provide feedback, but response rates were low.

2.3.3 Data analysis strategy

The PI group session was audio recorded and subsequently fully transcribed verbatim by an individual outside the primary research team. The transcription was checked by team

members for accuracy, and then key points were summarised for reference. As per Braun and Clarke (Braun and Clarke, 2006), a qualitative thematic approach to data analysis was used. Thematic analysis is a flexible methodology for determining themes in a quantitative dataset, and its subsequent analysis. It has evolved over the past 4 decades, and is now commonplace in analysis of such data, especially coming to prominence with the work of Braun and Clarke. Its use was determined here given the inherent flexibility of its approach to engage with such a dataset, particularly to identify and analyse the significance of themes. NVivo Pro version 12 (QSR International Pty Ltd), a qualitative analysis software package was used to assist with data management and narrative analysis. There are many similar programs available (Friese, 2019), although NVivo is the most widely used. This software aids researchers in determining themes inherent in the raw data, i.e., the transcript, and then developing themes, categorising data including with the use of coding, and then allowing for data visualisation. Nina Martin (NM), Leeds Beckett University, assisted in the transcription and coding of the transcript.

The six phases described in the Braun and Clarke method and how they relate to this dataset were used in a non-linear fashion using a data-driven and inductive approach:

1) “Familiarisation with the data”: several members of the team independently listened to the audio-recording and read the transcript several times. Initial observations regarding themes were noted, and a summary made
2) “Coding”: labels were generated for broad subjects and important features of the data. This coding was undertaken at a semantic and conceptual level to identify relevant, interesting, and suitable items in the data. Coding was checked by team members for accuracy and relevance, and all codes were collated.
3) “Searching for themes”: An iterative process then began to facilitate clustering of codes, and to investigate themes inherent. This also involved active discussion to bridge gaps of understanding and evolve consensus on themes.
4) “Reviewing themes”: The identified themes were reviewed to appreciate the key and subthemes in within those identified, and then discussed with the wider study team.
5) “Defining and naming themes”: A collaborative approach was taken to ensure concise but relevant themes were identified and defined.

6) "Writing up": The themes and narrative were collated in a study report that was circulated to the wider study team as well as the PI participants. This was peer-reviewed, edited, and amended after further expert opinion. Further critical review was provided by a participant from the PI group, who actively participated in editing and verification of the final report as is good practice and encouraged in PPIE guidance. This was subsequently published and is available via open access, in keeping with the spirit of PPIE in publicly sharing outcomes and knowledge (Parkash et al., 2021b).

2.4 Results

The PI exercise took place over the course of 3 hours at The University of York in a limited access, sound-proofed room, with all participants present throughout. Clinical researchers were present during the initial discussion as outlined in methods. By using the six phased method described by Braun and Clarke (Braun and Clarke, 2006) in analysing this data thematically, the following core themes were generated:

1. Assessing the quality of the participant-facing written material,
2. Improving the study design of both an uninfected sand fly biting study and a *Leishmania* CHIM study
3. Factors associated with involvement in the research, and potential motivations

These broader themes and individual subthemes are given in appendix Table 7.2, whilst a summary of the results are given in this section. Some of the included quotations have also been published elsewhere (Parkash et al., 2021b). The results were also reviewed by one of the focus group participants, Morgan Steigmann, (MS), prior to publication. MS was therefore included as an author on the published results.

2.4.1 Core Theme 1: Assessing the quality of the participant-facing written material

This theme centres on the significance of high-quality participant-facing materials to encourage recruitment and engagement with any future study, as discussed by the PI-group volunteers. Three themes were embedded within this core theme:

i) Need for clarity of message, ii) Consideration of the aesthetics of the proposed material, iii) Consideration of written content.

2.4.1.1 Need for clarity of message

This was the largest single theme, encompassing a number of sub-themes, with the central message of safety, study processes, induction and entry criteria. The study participants emphasized the need for increased clarity in the written materials aimed at volunteers, particularly concerning the nature of the health screening, the number and duration of study visits, and the perceived balance between time commitment and remuneration. Additionally, they required a better understanding of the inclusion and exclusion criteria, the potential for scarring after being bitten by a sand fly, the size comparison between the sand fly and parasite, potential for anaphylaxis from sand fly exposure (and relevance of different sand fly species), the management of any anaphylaxis reactions, the potential burden of disease, and the number of sand flies exposed to each participant and the nature of the bite experience that was expected. One participant cited a quick online search on sand fly reactions that revealed a suggestion of anaphylaxis associated with 'sand flies' to highlight the need for such clarity.

> *P7: Because I just Googled sand fly anaphylaxis and found Zane Mirfin writing about people, you know, I mean those aren't the sand flies you're using and it is completely anecdotal but if somebody's looking at this and they could do exactly that, Google 'sand fly' and 'anaphylaxis' it does pull up anecdotes.*

> *You need to be clear that with this strain [of sand fly], with this one, these things are not, there is no recorded incidence of that [recurrence] happening. You need to be really clear. (P9)*

Further clarification was sought regarding details of an infected sand fly biting study, including the standards of sand fly rearing, including risk of unintended transmission of pathogens. It was also suggested that any initial study should make reference to the overall

themes of the project to include the aims of the final study, to allow for a candid sharing of information.

Participants in the exercise asked for inclusion of future studies, even in a brief format, to be included in preliminary studies. This has particular relevance to the discussion of use of study outline of a formal CHIM, to be included in participant information leaflets about any non-infected sand fly biting study. The discussion also focussed on ensuring clarity around changes on acquisition of leishmaniasis through a CHIM, subsequent passage to others, and implications for longer-term immunity. A lengthy discussion also focused on potential treatment options following and infection. Concerns about safety following this then centred on provision of emergency clinical support, i.e. out-of-hours emergency contacts. The theme of importance of message also continued with a suggestion around terminology and choice of language, as well as visual formatting and layout of written information:

> *P7: And I think another thing with the literature was making it clear what the actual sort of, like the burden of the disease is. That it is this awful disfiguring disease you know and that's what we want to stop. You're not going to be getting that disease. What you're doing is developing a model to study it…*

The biting process also generated a discussion on use of specific terms that may hinder or attract recruitment, as discussed by one participant:

> *…saying you're going to have a feeding chamber strapped to your arm just sounds a little bit sinister. (P7?)*
>
> *P2?: I think feeding sounds a bit off-putting.*
> *P7?: Biting doesn't sound so bad because they are going to be biting you.*

2.4.1.2 Consideration of the aesthetics of the proposed material

This theme is mainly concerned with the selectin of images in participant facing material, namely those of the sand fly, of a cutaneous leishmaniasis lesion, and a representation of the scarring that can be expected following exposure to an uninfected sand fly bite as well as within a CHIM. The discussion also focused on use of such material in public facing recruitment drives, for example in print, posters and online features, in order to give an accurate representation to potential participants:

> *…it would be helpful if you put a photo to show of [the scar] afterwards. (P9)*

2.4.1.3 Consideration of written material

Many of the example materials, and indeed the consent for participants of this study contained substantial amounts of material related to General Data Protection Regulation (GDPR). The use of such material was questioned by participants, and it was suggested that an abridged version could be used to allow for better readability, with a link to online sources for a more expansive discussion. A plain English summary was suggested as the basis for any information leaflet to avoid inaccessible scientific terminology. Given the altruistic nature of volunteering for clinical studies, the suggestion was made that the impact that a volunteer should be emphasised, with relevance to increased scientific learnings, as described by one participant:

> *…when you talk about the potential benefits of the study I think you need to add on that one of the potential benefits is you get to make a contribution, you get to make a difference in helping us create a model that will develop a vaccine that will change and possibly save lives. (P9)*

2.4.2 Core theme 2: Improving the study design of both an uninfected sand fly biting study and a *Leishmania* CHIM study

The various treatment options for cutaneous leishmaniasis were discussed at length during a protracted discussion between clinical investigators and the public involvement group participants, as well as the proposed study design. Six themes emerged, with the main

theme being the appraisal of the 'bite site' location, available treatment options including oral tablet therapy, topical treatments, parenteral treatment (intra-lesional and intravenous therapies), cryotherapy (cold therapy), heat cauterisation, chemical cauterisation and surgical removal (including punch biopsy and excision biopsy). It was made explicit that treatments were not likely to be required in an uninfected sand fly biting study, although were expected in cutaneous leishmaniasis CHIM study following lesion development.

This core theme had six embedded themes: (i) Volunteer engagement, (ii) recruitment of volunteers, (iii) anatomical site for sand fly biting, (iv) treatments options in a cutaneous leishmaniasis CHIM study, (v) involving participants usual care practitioner in the decision to recruit, and informing of progress and (vi) time commitment

2.4.2.1 Volunteer engagement:

The PI consultation group participants discussed several methods that could be used to enhance recruitment to the study. This included study inductions held with multiple volunteers in a group setting, volunteer experience videos, online material and blogs. One participant suggested group activities would be beneficial in recruitment:

> *P7: Yeah, I think group settings are actually very good for generating discussion and people will ask questions you haven't thought of and you'll ask questions they hadn't thought of …*
>
> *P7:…again, if you had a website you could have stories you know about people and the effect that it has on people's lives.*

2.4.2.2 Recruitment of volunteers

PI group participants suggested a number of methods to assist in recruitment, including the use of electronic technologies such as email including targeted mail and newsletters, online sources and social media. The suggestion was that a combination approach could be effective with traditional offline methods such as radio adverts and newspaper adverts. Participants also suggested using platforms that allowed for interaction to use as both

educational resources but also to improve recruitment to studies. This could include surveys and other methods to automate the pre-screening process. One participant suggested social media was used by other centres:

> *P7: Facebook is one way that I know they've [other researchers] tried.*

2.4.2.3 Anatomical site for sand fly biting

The clinical research team suggested a range of potential biting areas, with that favoured being the area distal to the antecubital fossa, on the volar aspect of the forearm of the non-dominant limb. Participants voiced their favoured areas which considered the areas which were cosmetically favoured for scarring, and those which had a propensity to scar. Many of the participants suggested that a choice of bite sites would be an important consideration for participant autonomy including for determining acceptability of various sites for scarring:

> *P7: It's that thing about social acceptability is that lots and lots of people have got those vaccination scars on the tops of their arms and that's just completely normal whereas a visible scar here it's a bit, it's just sort of human beings our acceptance of scars.*

> *P5: I could make a suggestion you know our suggested site is here but if you like you can have it sort of elsewhere.*
> *P?: So, you have a choice?*
> *P5: Exactly, and ninety percent of the time they'll just go with what you suggest because they don't have a strong opinion they won't care. But if they do sort of feel very strongly, great they get to actually feel in control.*

2.4.2.4 Potential treatment options in a cutaneous leishmaniasis CHIM study

There are several treatment options in the management of leishmaniasis, and these were discussed at length with participants. This discussion included advantages and disadvantages of each option, and the schedule and mode of delivery of treatment. Participants suggested that use of excision biopsy, to remove localised skin lesions was the

favoured treatment modality. Many of the participants voiced that the rationale for this treatment choice would be a mix of being reassured that the lesion was removed, but also the altruistic aspect of donating a piece of tissue for further research and analysis, and to potentially gain novel insight:

> *P12: Would you think it was more reassuring to excise the lesion and use the ointment?...*
> *…P9: Yes.*

> *P1: So, if we kind of compromise and say from a study point of view our first choice is to take a biopsy so we can test it…*

> *P3: I wouldn't mind if I understood there was a benefit. If it says you'll have a biopsy and I had a large birthmark and the reason I wouldn't want excision is because having that off was quite unpleasant, but it was worth it for the biopsy results. I wouldn't have had it off as a cosmetic procedure. So, I think if you're advising people, we're taking it off by excision because we're going to do this to it rather than just for the sake of getting it*

> *P7: So, a small biopsy is, if that's part of the protocol. If you knew that was what you were signing up to. I mean I would be, personally I would be more than happy with that, you know, but that would be if it was what I had signed up to…*

2.4.2.5 Involving participants usual care practitioner in the decision to recruit, and informing of progress

Participants suggested that input from their General Practioner would be encouraged in order to confirm eligibility and to ensure accessible record-keeping within the NHS database:

> *P5: I think it's good [contacting the volunteer's GP] because for example with vaccine history a lot of people don't necessarily know exactly what they had vaccines for whereas you know that will be in their medical records.*

2.4.2.6 Time commitment

It was discussed with participants that volunteers in an uninfected sand fly biting study and a *Leishmania* CHIM study would require close monitoring in both the short and longer term. This would take the form of follow-up over the course of several months, but on the day of sand fly biting there would be a need to continue to monitor participants after the main intervention to look for evidence of any reactions, including anaphylaxis. Participants suggested continued observation was generally favoured, although they proposed as short a time as possible in order to make the time commitment acceptable:

> *P9: …as long as the two hours would be reassuring to me that, you know, most anaphylactic reactions would happen within that time frame then that would make me reassured and happy.*

2.4.3 Core Theme 3: Factors associated with involvement in the research, and potential motivations

Participants were asked about their motivations for involvement in the consultation exercise and if that translated into similar motivations for either the FLYBITE or the LEISH_Challenge studies. Three themes were generated within this overarching theme which included i) remuneration for time and involvement, ii) altruistic intentions, iii) and mixed motivation.

2.4.3.1 Remuneration for time and involvement

Participants suggested that financial reward played an initial part in attracting volunteers to the study, and was described in one case as a potential supplement to their regular income:

> *P5:…it's a very time effective way of supplementing their income. That's why everyone I know took part in them [i.e. clinical trials], chose to take part in them.*

2.4.3.2 Altruistic intentions

For many participants, altruism was an important aspect of the decision to take part, partly evidenced by the earlier discussion on excision biopsy. Participants stated explicitly, that they wished to 'make a difference' to vaccine and clinical scientific research:

> *P9:…I think there are a lot of people who want to get involved [in clinical trials] to make a difference as well.*

2.4.3.3 Mixed motivation

The majority of participants suggested the motivation for involvement in clinical studies would be both financial and altruistic, suggesting that both are important draws for inclusion:

> *P5: The altruism, it makes me feel better about taking part in it but it wouldn't have been enough by itself.*
> *P9: No, I think it's very much both. I think a lot of people are motivated by both of those things it's like I want to make a difference, I really want to make a difference but oh that's great if I actually get some payment too that's great. And I think one of the things when you talk about the potential benefits of the study I think you need to add on that one of the potential benefits is you get to make a contribution, you get to make a difference in helping us create a model that will develop a vaccine that will change and possibly save lives.*

2.5 Discussion

This PI activity delivered a range of public views that will inform further *Leishmania* CHIM development work (see later chapters). The PI group was conducted in an adaptive manner, which was unexpected, although this enabled full descriptions of the studies to be given, as well as improving the range of views voiced. The language of choice around CHIMs in general, and with relevance to clinical features of *Leishmania* management, required clarification for participants to comprehend the message. Clinical investigators were involved heavily in offering this debrief.

Material was shared with participants in advance (see appendix Figure 7.1 to Figure 7.8), including a plain language summary, and participant information leaflets. Participants therefore had the opportunity to dissect the information, and some brought prepared annotations to discuss the themes further. Use of terminology and accessible language was a common feature of the discussions, and participants emphasised the importance of include plain language, either as a summary within the document or indeed more simplified language throughout. Given the fact that 1 in 6 adults in the UK have reading level at or below that of an average 9-year-old (National Literary Trust, 2022), accessible language in science communication is a necessity (Judd and McKinnon, 2021; Koswatta et al., 2022), and indeed use of freely available editing tools to simplify are encouraged to improve accessibility of material (Hemingway, 2023). In the coming years, use of artificial intelligence-driven platforms may encourage further improvement in accessible material. The NHS and associated organisations have suggested guidance to improve such literacy (Hemingway, 2023; NHS Digital 2023). The aim is pubic and patient facing literature to feature a reading age of 9 to 11 years, although it is accepted that given the complexity of medical interventions, 11 to 14 years is acceptable in certain cases (NHS Digital, 2023). There is a need however to balance simplicity with brevity of message. Many of the participants craved additional insights into the underpinning science, demonstrating this. CHIM studies are unique amongst clinical research, including the mode of delivery and character of the 'intervention' and the ethical responsibility, and as such this balance is pertinent here. This is further complicated by the nature of a sand fly initiated *Leishmania* CHIM, which involves a neglected and not recognized disease, with potential for invasive intervention. Accessible interventions have been shown to have impact outside that of the initial study (Stricker et al., 2020).

Within the social sciences, reflexivity is an important concept concerned with examining the biases of contributing researchers, and their effect on the outcomes (Finlay, 1998). Its use and understanding can improve the reliability of information generated, through a dynamic process of evaluation of the research experience. The varied background of staff associated with many research studies, their prior lived experiences, experience of the research topic and awareness of their own biases, will all impact their evaluation and analysis of data, and

potentially the outcomes. As CHIM studies are complex and have multiple facets to consider including logistical and ethical concerns, it is imperative that reflexive practice is considered within this study, to ensure participants can engage with the material, and are allowed to express any concerns fully. The clinical researchers involved had no direct prior experience of CHIM studies, although did have both an awareness of CHIM studies, direct involvement in novel vaccine studies, as well as experience in the management of *Leishmania* and clinical research more generally. The research team, including the independent chair had a varied exposure to research, from an early career stage to experienced Professorial level experience of large multi-site international studies. This uniquely heterogenous mix of researchers inherently reduced the risk of any skew of opinions and therefore any research biases. This fostered an environment within the PI group that allowed for themes to be generated freely by participants, as well as for study design to be questioned.

The PI group activity generated many themes, although the most impactful were proposed amendments to the study design. At the outset of this process, it was mooted that a *Leishmania* CHIM would be sand fly-initiated, and termination of infection would likely be via use of systemic antileishmanial drugs, or localised antileishmanial treatments. There are no consensus UK guidelines on treatment of leishmaniasis, although the Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA) and the American Society of Tropical Medicine and Hygiene (ASTMH), are frequently used (Aronson et al., 2016). In clinical practice, treatment regimens are individualised and although based on guidance, are also based on clinician choice, drug availability and species of *Leishmania* involved (Parkash, Laundy and Durojaiye, 2023). As such there remains a degree of flexibility in treatment options. This flexibility and divergence in practice between clinicians and centres was discussed with participants. Excision based treatments, both full excision biopsies and punch biopsies may be used for early lesions but are not universally favoured by clinicians due to the additional scarring potential, and concerns with respect to residual parasite and subsequent potential for reactivation. The scientific merits of providing a biopsy for analysis were also discussed, including potential for learning about novel mechanism which may contribute to the understanding of leishmaniasis in general but also improve understanding of *Leishmania* vaccinology. This has been evidence by prior CHIM studies, for example the outcomes of a malaria CHIM which induced a change in future vaccine strategy (Sheehy et

al., 2012). After evaluating this information, participants suggested that that they favoured a biopsy/excision-based approach, which was unanimously agreed upon. The rationale given was that the opportunity to donate tissue for research was a motivating factor in volunteering, but also the psychological consideration for removal of parasites from affected skin. The psychology of infection with an infectious disease and psychogenic response to the inoculum is complex and attempts at its characterisation has been attempted for at least a century (Klauder, 1936). Parasitic infection is very evocative, particularly those that are skin-transmitted, and therefore fits with the suggestion for biopsy in this participant group exercise (Kupfer et al., 2021; Blake et al., 2016). Use of skin excision biopsy is not a new concept in skin-based controlled human infection studies. Skin biopsy has been used to date in CHIM studies for BCG and dengue virus (Carter et al., 2023; Choy et al., 2022), and in a malaria study using explanted skin, biopsy was used to determine characteristics of the sporozoites after skin injection (Winkel et al., 2019). Skin biopsy has also been mooted for use in a future human infection study for *Mycobacterium ulcerans* (Muhi et al., 2023).

Prior to the development of this novel CHIM, biopsy was not considered a possible treatment option within a *Leishmania* CHIM due to a mixture of expected poor participant acceptability, and no clear consensus for its use in clinical practice. Participants from the PI group agreed that any lesion developing following exposure should reach the smallest possible diameter to allow for an accurate diagnosis, including incidental causes, but also to provide enough tissue for useful analysis. These outcomes then prompted detailed discussion with infection and dermatology clinicians, concerned with the regular care of patients with cutaneous leishmaniasis. This expert group agreed with surgical excision as a valid method to terminate lesions in the context of this CHIM. Biopsy as a treatment for leishmaniasis has now been incorporated into the CHIM protocol (see chapter 4) - a major difference in the previous suggested approach, brought about by this public involvement process.

The limitations of this study centred mainly around representation and diversity. Several attempts were made to broaden the group of participants to have a more representative sample population through engaging different groups and through circulating

advertisements widely. Although ethnicity and age was not specifically recorded a lack of ethnic diversity amongst the PI consultation group participants was noted. Previous studies at the University of York have also encountered similar issues and reflects local demographic indices. This has also been observed in many other studies and trials for many years, with efforts to improve recruitment not reflected in the makeup of study volunteers (Oh et al., 2015). Recruitment for large studies such as the COVID-19 UK clinical trials, also suffered from lack of diversity with relevance to Asian, Black and Mixed ethnic groups (Murali et al., 2023), compounded by lack of vaccine uptake in these groups during UK national vaccination campaigns on a background of disproportionate poor health outcomes from COVID-19 disease (Hussain et al., 2022). Although this PI consultation group was designed to consider studies to be conducted in the UK, this lack of representation within the PI group may not represent accurately the range of patients who may sufferer from leishmaniasis in resource poor environments. To adapt this to a LMIC, further public involvement work in new regions might be required as seen in other CHIM studies (Elliott et al., 2018; Jamrozik and Selgelid, 2020a, 2020b; Njue et al., 2018a).

Leishmaniasis infection may have differing outcomes in those of darker skin types, with one study suggesting that this is further compounded by people of colour having disparities in access to healthcare (Bruhn et al., 2018). Distinct skin phototypes may also have differing responses to *Leishmania* infection, potentially resulting in increased scarring and other skin implications such as hyper- or hypopigmentation (Kumar, 2015). Increased ethnic diversity within the PI group may have resulted in increased recognition of these factors, although one of the participants commented that they had '*mixed race children and my husband… he scars, you know'*. Most of the PI group participants were recruited from the local York area and had a history of attending further education. In addition, York is considered one of the least deprived regions of the UK and so motivations for involvement in this study may not reflect those of other participant groups (Gov UK, 2020).

Although attempts were made to recruit a patient who had previously undergone treatment for cutaneous leishmaniasis, this was not possible due to availability and poor response rate. Given the predominant cases of imported cutaneous leishmaniases to the UK are caused by New World *Leishmania* species*,* this may have in fact complicated discussions (Marks et al.,

2016; Wall et al., 2012; Parkash, Laundy and Durojaiye, 2023). Given Old World disease can cause more aggressive and persistent lesions that require prolonged treatment (Parkash, Laundy and Durojaiye, 2023) this is distinct from the usual pathogenesis of *Leishmania major*, the proposed challenge agent for the *Leishmania* CHIM. This would be reinforced by the nature of the treatment options given in other species of *Leishmania*, which would invariably give a differing experience (Mcgwire and Satoskar, 2014). Science literacy in relation to recruitment for studies is a difficult balance between accessible language and ensuring true understanding and therefore informed consent language recruitment, although it is feasible that improved language around such studies may strengthen recruitment and increase the reliability of results (Judd and McKinnon, 2021; Koswatta et al., 2022).

It has been increasingly noted that to assist in carrying out good quality and reliable research, public involvement is an invaluable tool. Its usage is variable and differ depending on experience of researchers, and local infrastructure to assist in its delivery (Thornton, 2008; Thompson et al., 2009). Even where support for the PI activity is well ingrained in research culture, the outcomes can be variable (Gray-Burrows et al., 2018). This contrasts with the experience discussed here, with the development of the Leishmania CHIM not possibly in the current format without the use of PI. Given the increase knowledge and awareness of both CHIM and PI, it is in inevitable that more studies will be developed in the coming years, as evidence recently by broad discussions around SARS-CoV-2 CHIMs (Piggin et al., 2022; Barker et al., 2022; Jamrozik et al., 2021). The themes from such studies do broadly mirror this study, namely subjective and objective risk to volunteers, appropriate remuneration, support for participants during the study and clarity of information (Gbesemete et al., 2020). Acceptability of CHIM studies to the public outwith study participants, following ethics committee approval is increasingly discussed and is an approach taken within this study (Jamrozik and Selgelid, 2020a; Jamrozik et al., 2021). This is evidenced by the planned continuous review using participant involvement throughout the development process in a lead up to a formal *Leishmania* CHIM, and following its conclusion (see Figure 1.6). This final PI activity will aid in establishing this CHIM as a standard model for evaluation of candidate leishmaniasis vaccines.

The future progression of this CHIM, if successful, may involve carrying out further CHIMs in endemic regions. There has been many discussions to-date about the potential harms and benefits of such an approach, many of which broadly mirror the findings in this study (Jamrozik and Selgelid, 2020a). Some other studies about motivations for inclusion of volunteers in CHIM studies have also discussed the difficult balance of altruism and the objective risks of challenge agents (Rose et al., 2021). To date however there has been very little discussion elsewhere about the involvement of participants in treatment options in CHIM studies, as observed here.

2.6 Conclusion

This description is the first detailed appraisal and discussion of public involvement in controlled human infection research for cutaneous leishmaniasis. Although recent public health campaigns have bought awareness, CHIM studies are still poorly understood by the general public. Better use of technology and digital platforms is necessary to reach more audiences as potential volunteers, but also to assist in maintaining engagement during a study. The major outcomes include improved participant-facing literature, and a better understanding of public perceptions of such studies. Moreover, the design, treatment and practical considerations of the future studies were positively impacted as a result of this exercise. There are chronic issues with diversity of participants at all levels of clinical studies, however greater awareness of these disparities will serve to find way to improve representation. Given the inherent bias of researchers in general, such studies are imperative in ensuring a balanced viewpoint and better accountability particularly with a view to carrying out future CHIMs in endemic regions.

3 Chapter 3: A clinical study to optimise a sand fly biting protocol for use in a controlled human infection model of cutaneous leishmaniasis

3.1 Introduction

3.1.1 Skin and vector-based controlled human infection models

For several vector-borne diseases where there is an unmet need for vaccines, controlled human infection models (CHIM) have been used to evaluate new treatments and vaccines whilst also allowing for exploration of pathogenesis (Payne et al., 2017; Darton et al., 2015). Such models have the advantage of planned follow-up from time zero of inoculation, until development of suitable pre-determined timepoints to fully characterise disease development. For many vector-borne diseases, prevention of new infections has focused on vector-based solutions. The World Health Organization has set out a vector control response for major vector-borne diseases to strengthen control efforts, although the targets are ambitious and do not focus on vector elimination (WHO et al., 2017). There has been a long history of deliberate human exposure to vector-borne diseases, using the respective natural vector, direct inoculation or in some cases a novel vector (see Chapter 1). Notable studies involving the natural vector include mosquitos for malaria (Talley et al., 2014), yellow fever virus (Clements and Harbach, 2017; Lederer, 2008) and dengue virus (Balasingam and Wilder-Smith, 2016), *Chrysops* and filariasis (Nutman, 1991), and now sand flies transmitting leishmaniasis are all diseases for which human infection studies have been in development (Jamrozik and Selgelid, 2021). Hookworm, schistosomiasis and tularaemia human infection studies draw parallels to leishmaniasis controlled human infection, given that there is a parasitic skin-delivered exposure approach that mimics the natural infection cycle. However, there is no specific vector that is likely to be used in human studies for hookworm (Alabi et al.), and a current CHIM protocol for schistosomiasis (Langenberg et al., 2020) abrogates the need for the aquatic snail vector. A historic tularaemia experimental infection study used skin inoculation and did not require the tick vector (Sawyer et al., 1966; Saslaw et al., 1961). Skin focused controlled human infection models where there is no specific insect vector include those for *Haemophilus ducreyi* (Brothwell et al., 2020; Janowicz et al., 2009) and

Bacillus Calmette-Guérin (BCG) which will be used to test *Mycobacterium tuberculosis* vaccines (Harris et al., 2014). There are some CHIMs developed for diseases of importance to skin infection, although the infection model concentrates on infection in other anatomical regions, including for *Streptococcus pyogenes* (Osowicki et al., 2021) and *Streptococcus agalactiae* (Clinicaltrials.gov, 2023). Future mooted CHIMs involving skin exposure or inoculation include those for *Staphylococcus aureus* (Welling et al., 2019; Howden et al., 2023), *Mycobacterium ulcerans* (Muhi et al., 2023) and babesiosis (Al-Nazal et al., 2022). A CHIM for zika virus has been discussed at length, although there is no consensus on whether a vector-delivered approach might yield better outcomes, however needle challenge is likely to be the first step (Palacios and Shah, 2019; Durbin and Whitehead, 2017).

Existing studies of skin infection usually make use of mouse models, and sometimes excess human material obtained from cosmetic procedures. There has also been some discussion of the use of bioengineered skin models (Low et al., 2020) and skin organoids (Sun, Zhang and Li, 2021). There is a varied response from human skin depending on the pathogen involved, and these models therefore have limited applicability to all skin diseases and skin inoculation models. There are significant structural and molecular differences between murine and human skins, for example their relative gene expression (Gerber et al., 2014). Typical structural differences include mouse skin being less thick (with relevance to dermis and epidermis) and having a larger number of hair follicles, and human skin having a more irregular boundary between the dermis and epidermis (Lynch and Watt, 2018). In terms of immunology, differences exist both in systemic and cutaneous immunity. Mice have a greater representation of bronchus-associated lymphoid tissue, which is not widely observed in humans as well as differences in lymphocyte expression. With respect to skin, T-cell and chemokine expression is distinct, including in their anatomical distribution. Further differences at the level of the skin include innate protection mechanisms, including the microbiome (Zomer and Trentin, 2018; Lynch and Watt, 2018; Gerber et al., 2014; Park and Im, 2020). See also Chapter 1 for further discussion of skin immunology with respect to cutaneous leishmaniasis. As well as the regional disparities that exist in health and impacts from climate change where many infectious diseases are present, there are also differences in skin type, melanin content and melanogenetic mechanisms between different

populations. This will be relevant to existing skin libraries that are used to test treatments, especially in relation to the skin of peoples who are affected by disease typically occuring in the Global South.

3.1.2 Sand flies as vectors in human infection studies

Novel findings have recently emphasized the importance of sand fly-derived factors that contribute to infection and pathogenesis. These include sand fly salivary gland proteins (Cecílio et al., 2020a), promastigote secretory gel and other parasite by-products (Lestinova et al., 2017), virulence factors (Atayde et al., 2016), exosomes (Atayde et al., 2015) and the microbiome (Dey et al., 2018b). The understanding of the impact of these factors on therapeutic efficacy of novel and existing treatments is developing (Kaye et al., 2020). The importance of the natural vector is typified by the observation of vector transmission facilitating infection or altering immune responses in a manner not seen with direct challenge, for example when using needle-challenge (Peters et al., 2012a; Dey et al., 2018b; DeSouza-Vieira et al., 2020).

Utilisation of sand flies both infected and uninfected, is not a new phenomenon in experimental human infection studies (see chapter 1). However, there is a need to have a safe and efficacious method that can be reproduced to allow for harmonisation across studies. A series of studies have demonstrated safety of controlled human exposure to sand fly biting (Luz et al., 2018; Vinhas et al., 2007; Oliveira et al., 2013), and a recent NIH protocol for sand fly biting on humans has been developed as part of a study to examine immune responses (Luz et al., 2018).

3.1.3 Protocol development

To develop a CHIM for cutaneous leishmaniasis, a series of underpinning studies were required (see Figure 1.6). In Chapter 2 I described a public involvement group exercise with participant preference for study procedures taken into account when developing protocols associated with the CHIM development. This has culminated in the protocol described in the methods section in 3.2.

The basis for the use of deliberate infection of humans with *Leishmania* stems from observed immunity seen with the centuries old Leishmanization practice, and subsequent human experimentation using sand flies and/or *Leishmania* parasite (discussed in Chapter 1 and earlier in this chapter).

Other groups such as at the NIH have considered use of a custom biting sand fly biting chamber to deliver uninfected sand fly bites to human volunteers (Clinicaltrials.gov, 2020). The exclusion criteria for this study included use of serum IgE, prior expose to sand fly salivary proteins (determined by screening assay), and exaggerated reactions to insect bites.

These prior experiences have allowed the development of the protocol described here, in a study which is known as FLYBITE.

3.1.4 Sand fly salivary gland protein

Given the known protective effects of sand fly salivary gland antigen on exposed participants against later exposure to *Leishmania* parasite, it may be important to understand the baseline serostatus of study participants (Aronson et al., 2021; Laurent et al., 2013; Aoki et al., 2022; Oliveira et al., 2015; Lajevardi et al., 2022). Although the mainstay of determining prior exposure to sand flies is by thorough clinical history, serological analysis may be a useful adjunct. Although it is known that salivary gland proteins enhance infectivity when co-delivered with *Leishmania* parasite (Ockenfels, Michael and McDowell, 2014a), there is a protective effect against later parasite exposure, which is largely mediated through delayed-type hypersensitivity (Belkaid et al., 2000; Oliveira et al., 2013). In addition, where disease does occur prior sand fly exposure is associated with milder disease (Mondragon-Shem et al.). Measuring of baseline serostatus was undertaken by specific anti-saliva IgG for both *Phlebotomus papatasi* and *Phlebotomus duboscqi* by enzyme-linked immunosorbent assay (ELISA) (see Methods section 3.2).

3.1.5 Outcome measures and study objectives

Primary Objective

The primary objective was the development of a sand fly biting protocol using pathogen-free sand flies which was effective and safe for volunteers. Volunteers aged between 18-65 years were to receive a bite or bites by sand flies using a watch-like biting chamber placed on the arm. The use of biting chambers containing 5 sand flies maintained on the arm for 30 minutes were evaluated, with the two sand fly species *Phlebotomus papatasi* and *Phlebotomus duboscqi* fed on (non-human) blood in the laboratory prior to human exposure.

Outcomes

Effectiveness will be assessed by counting the number of sand fly bites immediately after the biting procedure using a dermatoscope, number of sand flies that demonstrate engorgement and persistent sand fly bite-related lesions on study follow-up. Safety will be measured by assessing adverse event data collected through history, clinical examination, blood tests and through subjective participant diary card data.

3.1.6 Secondary Objectives

1. To determine human response to sand fly bite for (i) macroscopic, (ii) dermoscopic, (iii) immunological, and (iv) haematological / biochemical outcomes.

2. Determine attitudes to sand fly bite by way of post-study focus group with study participants

Outcomes

1. These are defined by (i) clinical photography using digital photography, (ii) dermoscopy, (iii) total IgE and the development of an antibody response to sand fly salivary gland proteins, and (iv) full blood count, and a full biochemical screen

2. Inductive content analysis of the focus group transcripts

3.2 Methods

3.2.1 Recruitment of volunteers and pre-screening

Healthy volunteers were recruited by advertisement (see recruitment poster, appendix Figure 7.10) within the University of York as well as local publications circulated to the local community, and both internal and external websites (LEISH_Challenge, 2023). Further recruitment took place across the University of York and included use of posters and a university-wide email newsletter. Social media was not used for recruitment. Recruitment was coordinated by myself, with assistance from a study project manager and research nurses. Participants registered interest by emailing the study coordinators as per advertisements, or by phoning the study office line. They were then given a copy of the participant information leaflet (see appendix Figure 7.11). Screening was a 2 step-process comprised of a pre-screening which could take place face-to-face or via telephone and then a formal in-person screening session. An initial pre-screening questionnaire was used to collect basic contacts details, and to assess eligibility, (see appendix Figure 7.9). Interested parties were given more information on the study, and the following questions were asked:

- age between 18 – 65 years old
- available for the duration of the study
- any history of *Leishmania* infection
- any history of travel within the last 30 days or for 30 continuous days to regions where Leishmania major-transmitting sand flies are present (This refers to regions where Leishmania major-transmitting sand flies are endemic including (but not limited to) the Middle East, Sub-Saharan Africa, and Asia)
- any current chronic illness requiring hospital specialist input or any other significant ongoing medical condition
- any allergies to medications or other allergens.

There was a minimum of 24 hours between pre-screening and formal in-person screening to allow participants to fully consider their participation, although pre-screening took place between 40 and 14 days prior to sand fly biting.

Professor Charles Lacey was the Chief Investigator for this study entitled FLYBITE, and I was a Principal Investigator, leading on the clinical aspects and overall running of the study.

3.2.2 Screening

The screening visits were in-person visits and took place between 30 & 7 days prior to sand fly biting. Participants were enrolled (sand fly biting) exclusively by me. A further discussion about the overall study aims, purpose, procedures, and potential risks was had. Participants were encouraged to ask questions during this process, and throughout the study. If participants were deemed suitable for inclusion and agreed to participate in the study, then informed consent was undertaken (see appendix Figure 7.12). At the screening visit the subjects underwent a full history, examination and blood testing as detailed in the case record form (CRF). Participants were asked about their past medical history, with particular relevance to any history of skin disease, atopy, allergy and anaphylaxis. A thorough travel history was also obtained. Physical examination included routine observations (blood pressure, heart rate, oxygen saturations, respiratory rate and temperature), and then a full systems examination focusing on cardiovascular, respiratory, abdominal, neurological and lymph node examination. A further focused examination of the participant's skin was undertaken including at the proposed sand fly bite site. The following domains were commented on:

- Inflammatory skin conditions
- Scarring
- Xeroderma (Skin dryness)
- Erythema
- Swelling
- Excoriation
- Ulceration
- Blistering
- Abnormal pigmentary change

This mirrored the examination during follow-up visits.

- Blood tests were taken at this visit for the following:
- Full blood count
- Urea and electrolytes

- Liver function test
- C-reactive protein
- Serology for blood-borne virus testing (Human immunodeficiency virus, Hepatitis B, Hepatitis C)
- Total immunoglobulin E (IgE)
- Peripheral blood mononuclear cells
- Sand fly salivary gland antigen ELISA
- rK39 *Leishmania* antibody test

At this visit a provisional date and time for the sand fly biting visit was agreed. Consent was obtained to contact the participants GP to confirm that the participant had not recently participated in any other clinical trials, that they had no significant ongoing illness, and there was no other concerns that the GP had prior to entry into the study (see appendix Figure 7.13). Once the results of all screening tests and the GP report had been received the eligibility was confirmed by the clinical team led by me. During the screening visit the volunteers were asked to provide their National Insurance or passport number so that this could be entered on to a national database which helps prevent volunteers from participating in more than one clinical trial or study simultaneously or over-volunteering for clinical trials (www.tops.org.uk). This included having a photograph taken which was be placed only in the in the participant's file.

3.2.3 Inclusion and Exclusion criteria

FLYBITE Inclusion criteria:
Participants had to fulfil the following criteria:

- Healthy adults aged 18 to 65 years on the day of screening
- Willing and able to give written informed consent
- Willing to undergo a Hepatitis B, Hepatitis C & HIV test
- Willing to undergo a pregnancy test during screening and follow-up visits and must not be breastfeeding (female volunteers)
- Willing to refrain from blood donation during the study
- Using a reliable and effective form of contraception (female volunteers)

- Judged, in the opinion of a medically qualified Clinical Investigator, to be able and likely to comply with all study requirements as set out in the protocol
- Without any other significant health problems as determined by medical history, physical examination, results of screening tests and the clinical judgment of a medically qualified Clinical Investigator
- Available for the duration of the study
- Willing to refrain from travel to regions where Leishmania-transmitting sand flies are present, from recruitment until the last study visit.
- Willing to consent to a report from the volunteer's GP confirming medical eligibility, to be provided before study entry
- Agree to registration on a national database of trial subjects to prevent over-volunteering (TOPS) which includes the taking of a photograph to be kept at the study site

FLYBITE Exclusion criteria

The volunteer was excluded from the study if any of the following applied:

- Receipt of a live attenuated vaccine within 30 days or other vaccine within 14 days of screening
- Administration of immunoglobulins and/or any blood products within the three months preceding the planned study.
- History of allergic disease/atopy or reactions or a history of severe or multiple allergies to drugs or pharmaceutical agents
- Any significant chronic skin condition as judged by the medical team
- Any history of Leishmaniasis
- Any history of travel within the last 30 days to regions where Leishmania major-transmitting sand flies are endemic*.
- Any past history of more than 30 contiguous days stay in regions where Leishmania major-transmitting sand flies are endemic*.
- Any history of severe local or general reaction to insect bites, defined as
- Local: extensive, indurated redness and swelling involving most of the antero-lateral thigh or the major circumference of the arm, not resolving within 72 hours

- General: fever ≥ 39.5°C, anaphylaxis, bronchospasm, laryngeal oedema, collapse, convulsions or encephalopathy within 48 hours
- Any history of anaphylaxis
- Female participants: pregnancy, less than 12 weeks postpartum, lactating or willingness/intention to become pregnant during the study and for 3 months following the study.
- Any clinically significant abnormal finding on screening biochemistry or haematology blood tests or urinalysis
- Total IgE levels >81 KU/L
- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months
- Tuberculosis, leprosy, or malnutrition
- Any chronic illness requiring hospital specialist input
- Any significant psychiatric conditions
- Any other significant disease, disorder or finding, which, in the opinion of a medically qualified Clinical Investigator, may either put the volunteer at risk because of participation in the study, or may influence the result of the study, or the volunteer's ability to participate in the study
- Unlikely to comply with the study protocol
- Participating in current or recent research within the past 3 months (as judged by study investigators)

*This refers to regions where *Leishmania major*-transmitting sand flies are endemic including (but not limited to) the Middle East, Sub-Saharan Africa, and Asia.

3.2.4 Study design

The study was conducted in the Translational Research Facility at the University of York, UK and was a non-randomised, participant-blinded clinical study in healthy participants with two parallel arms and a 1:1 allocation. 12 participants were recruited to two study groups, exposed in a 1:1 allocation to either *Phlebotomus papatasi* or to *Phlebotomus duboscqi* sand

flies. Allocation to each group was determined by participant availability, and coincidental availability of sand fly species. Although other vectors have shown to demonstrate competence for *Leishmania major* transmission, both *P. papatasi* and *P. duboscqi* are recognised to be the major natural vectors for *L. major*, the species of *Leishmania* that is included in the controlled human infection model described in chapter 4 (Giraud et al., 2019a; Ferreira et al., 2022; Cecílio et al., 2020b). To-date limited studies have demonstrated rate of clinical infection development, transmission competence or biting rate in human studies between these vectors.

Sample size was determined to allow balance between safety and efficacy and to allow outcome measures to be elucidated. As this is an initial study, the data on take rates and lesion development will help to inform sample size calculations for future studies. This is on a background of CHIMs expected to generally have lower sample size in comparison to conventional clinical trials (Coffeng et al., 2017), particularly with relevance to experimental *Leishmania* human infection (Mohebali, Nadim and Khamesipour, 2019a). Generally, the anticipated take rate and the expected effect size of the intervention is considered when determining take rate, for which clinical data in *Leishmania* is lacking (Balasingam and Wilder-Smith, 2016). Other similar CHIMs have employed low numbers of participants in the pilot and early studies. In the initial pilot studies for malaria, 4 and 5 participants took part respectively (Herrington et al., 1988; Cheng et al., 1997) and in a Schistosoma CHIM, initially 3 participants were exposed to cercariae prior to further participant intervention and dose escalation (Langenberg et al., 2020). Previous *Leishmania* studies suggest the take rate in a controlled setting was high, at 82.6% (Khamesipour et al., 2005a) and when used for leishmanization, the take rate was higher (Mohebali, Nadim and Khamesipour, 2019a). Sand fly biting rate varies according to geographical location, species, environmental factors, as well as a host of other factors, although there are few studies that explore bite rates on humans (Vojtkova et al., 2021; Cameron et al., 2016). Therefore, in determining the sample size in a parallel two arm study with each of the vectors, 6 participants in each study arm, was determined to be adequate in achieving the aims of this study. A 1:1 allocation of the biting aperture size to either 6mm or 8mm was determined in each vector intervention arm.

The study was conducted according to the principles of the current revision of the Declaration of Helsinki 2008 and ICH guidelines for GCP (CPMP/ICH/135/95). All participants provided written informed consent for the sand fly biting study and the focus groups prior to enrolment.

3.2.5 Sand fly maintenance and use in study

Leishmania-free *Phlebotomus papatasi* and *Phlebotomus duboscqi* sand flies were used. The sand fly colony is maintained at Charles University, Prague. This is an established colony and is widely used in research studies involving sand flies given the significant expertise at this unit (Volf and Volfova, 2011), (Lawyer et al., 2017). Sand flies were transported via specialised courier to the Department of Biology, University of York in specialised containment from the Charles University, Prague. Air freight was used to ensure a timely and reliable shipment time in order for sand flies to at the correct stage of development to allow biting to occur, as the life span of these species of sand flies from egg to adult is a few weeks to a month (Cecílio, Cordeiro-da-Silva and Oliveira, 2022; Volf and Volfova, 2011). Clay is placed in the bottom of the transport unit to help maintain humidity. The sand flies are shipped between days 3 to 5 of reaching the adult stage of development, as holometabolous adult insects.

On arrival the non-infected sand flies were kept within a secure insectary at the University of York, with several layers of controlled access to a sealed room to prevent escape of flies. The room is negatively pressured and employs an air curtain at the entrance to prevent escape of sand flies. The room also contains insect electrocutors as a further safety measure. There is a double-door system to ensure that any further exposure is limited. No *Leishmania* experiments including with other animals take place in close proximity to this unit. All users of this facility wear white overalls that can be easily checked for sand flies prior to leaving and are removed upon leaving the unit. The sand flies are kept in a dedicated incubator (26°C, 70% humidity, photoperiod of 12 hours light and 12 hours dark). They are housed within a 40cm^2 nylon insect cage with a feeding membrane (BugDorm, MegaView Science Co., Ltd., Taiwan) inside the incubator, and taken out for feeding and experiments. Sand flies are maintained on a sugar solution between feeding (soaked cotton

wool, 50% sucrose solution). Only trained members of staff with health clearance and appropriate experience were allowed contact with the sand fly storage facility.

The sand fly colony was screened for phleboviruses and the Flavivirus genus. For Flavivirus testing, viral RNA was isolated and then genomic DNA was removed to avoid false positives. A conserved region of the NS5 gene was used as a PCR target. For the Phleboviruses a RT-PCR for Sandfly fever Sicilian virus group, Toscana virus, and Massilia virus was conducted.

When sand flies were aged 5 to 7 days in the adult phase, and 18 to 25 hours prior to a blood the sucrose solution was removed to starve them and encourage later feeding. A membrane feeding system was subsequently used to provide a blood meal using rabbit blood (Hemotek membrane feeding system). Female sand flies prefer to feed in the dark, hence the insect cage is covered with an opaque material to mimic the natural dusk feeding habits of phlebotomine sand flies (Killick-Kendrick, 1999). Feeding took place for up to 1 hour, with male sand flies present. Although only female sand flies transmit the *Leishmania* parasite and take a blood meal to facilitate oviposition, the presence of male sand flies increases the rate of feeding (Lawyer et al., 2017). The sand flies were ready for further biting on human volunteers around 12-15 days post blood meal, at age 18-21 days of the adult form.

Following the blood meal, evidence of feeding is determined by sand fly engorgement. If the sand flies do not demonstrate adequate feeding based on engorgement, then the study is postponed, and participants are re-booked based on future sand fly availability. Sand fly handling takes place after sand flies are exposed to low temperatures to reduce their metabolic activity, and then sand fly containment boxes are placed on ice to maintain low temperatures (Benkova and Volf, 2007). Once metabolic activity is reduced, sand flies can be handled using fine tweezers, and then placed in a custom watch-like sand fly biting chamber.

3.2.6 Sand fly biting chamber device

The sand fly biting chamber (Precision Plastics Inc, Maryland, USA) is composed of clear acrylic and has a containment unit for sand flies, akin to a watch face, which is 3.68cm in diameter, and 3cm in depth, with edges for straps out to 6.35cm (see appendix Figure 7.14). Adjustable Velcro straps hold the sand fly biting chamber in place during use. The total internal volume for sand fly containment is 9.68cm^3. The underside of the chamber is in contact with the participant's skin, and there is an inferior opening over which a piece of nylon gauze can be placed and secured. Sand flies can bite through this nylon gauze but are unable to escape. A further piece of filter paper is used to restrict sand fly biting to a predetermined size (sand fly biting aperture). On the superior aspect of the chamber there are a number of holes to allow for ventilation, 4mm to 4.5mm in diameter. Wire gauze is affixed in these holes to prevent escape of sand flies (see Figure 3.1).

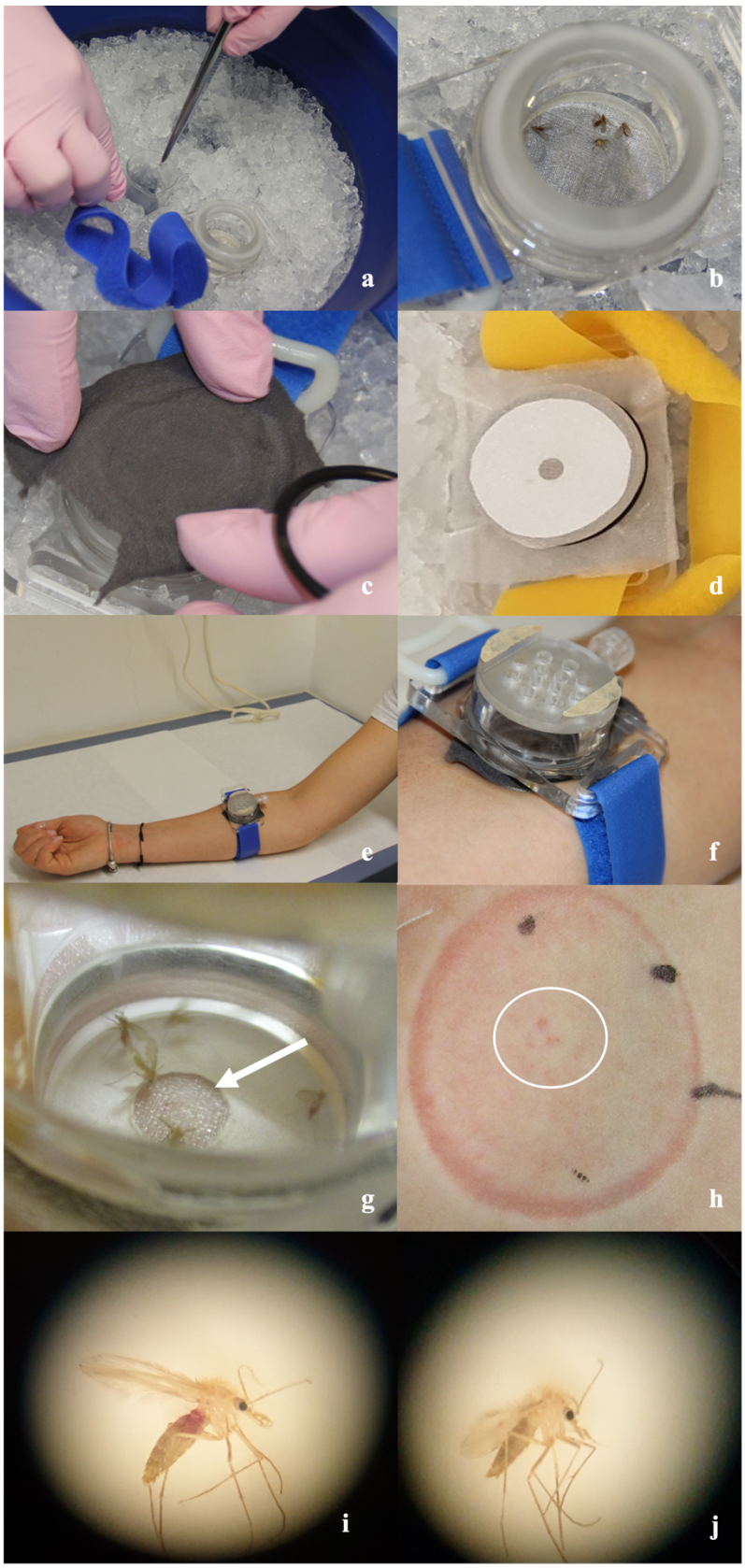

Figure 3.1: Sand fly biting chamber and study procedures

Main steps in the sand fly biting procedure. (a & b): 5 sand flies are placed inside the sand fly biting chamber. Fine tweezers are used to handle them. The sand fly biting chamber is

kept on ice to reduce sand fly metabolic activity to allow ease of handling. (c) A gauze filter is used to cover the underside of the biting chamber to prevent escape of sand flies but to allow sand fly biting to take place. (d) Filter paper with a hole (aperture) is used to form an opening of between 6-8mm to limit the area for sand fly biting. (e & f) An adjustable Velcro strap is used to ensure an appropriate fit. The biting chamber placed approximately 3–4cm distal to the antecubital fossa. (g) Sand flies within the biting chamber; biting aperture with gauze visible in the central portion (arrow). (h) Participant skin demonstrating pressure mark from biting chamber and small visible bite marks (circled). (i & j) Sand flies pre- and post- biting. (j) following biting a red swollen abdomen is visible. This is evidence of a blood meal having been taken.

Adapted from a figure produced for (Parkash et al., 2021a).

3.2.7 Sand fly biting visit

Participants were invited to attend the Translational Research Facility at the University of York on the proposed day of sand fly biting. A neutral non-scented skin wash was given to participants to reduce host pheromones, and other chemicals at the level of the skin which could introduce variability of biting behaviour of sand flies (HAMILTON and RAMSOONDAR, 1994)',(Rebollar-Tellez, Hamilton and Ward, 1999)',(Nevatte et al., 2017). The vector used on the day of biting was based on availability, sand fly survival rates and success of the initial blood meal. The sand fly biting chamber was loaded with 5 female sand flies up to 30 minutes prior to the biting intervention start time. The biting chamber was transported to the clinical area inside 2 larger containers to prevent any issues with sand fly escape. The participant's hand dominance was recorded, and a recommendation to use the sand fly biting chamber on the non-dominant hand was taken up by all participants. The two potential areas for placing the sand fly biting chamber were the upper inner arm above the medial epicondyle or the volar aspect of the medial forearm (2-3cm distal to the antecubital fossa). All participants were encouraged to use the medial forearm based on risk of scarring and ease of visualisation for follow-ups and reporting. The participants understanding and willingness to partake in the study was reviewed prior to the intervention. A further clinical examination including baseline observations took place. The sand fly biting chamber was placed on the participant's arm for 30 minutes. Pain and itch were recorded on a visual analogue score (VAS, 0-10 scale) 15 minutes after biting, along with any subjective biting sensation experienced by the participant. I observed the participant throughout this time along with a research nurse. Following sand fly biting, and removal of the sand fly biting chamber. Participants were observed for a further 2 hours. Every 30 minutes an evaluation of baseline observations, a further VAS for pain and itch, a dermatoscopy review of number of visible bites (MoleScope II – Mobile Dermatoscope attached to an Apple iPhone 7), and a score of 0-3 of clinician recorded erythema, swelling and blistering was undertaken. Sand flies were also evaluated for visual engorgement, and further dissection took place to determine microscopic evidence of blood meal. The schedule of visits is shown in Table 3.1 and Figure 3.2.

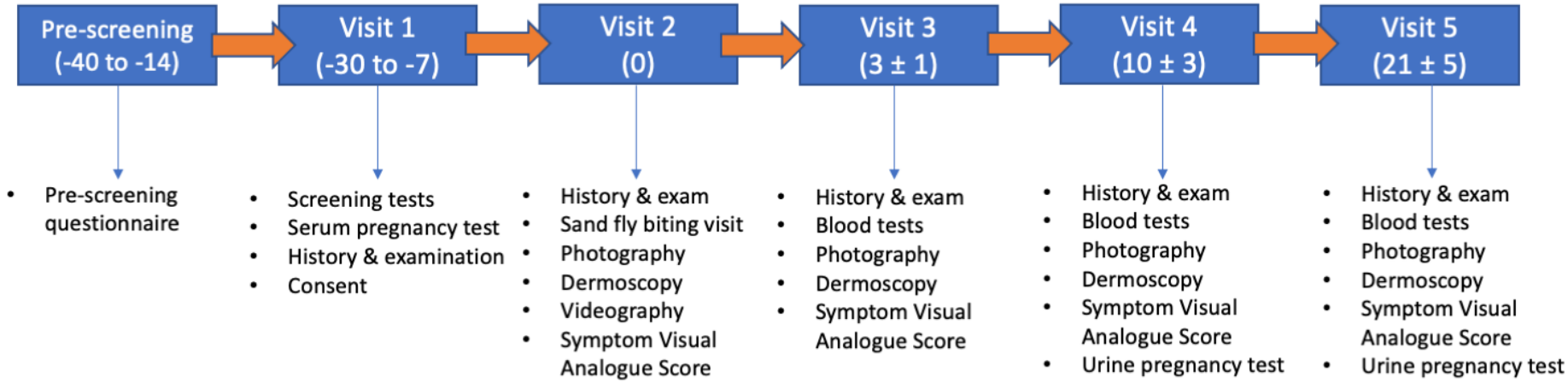

Figure 3.2: FLYBITE Schedule of study visits

Adapted from a figure produced by me for (Parkash et al., 2021a).

3.2.8 Follow-up visits

Further visits took place at 3, 10 and 21 days after the biting visit. The windows of compliance with the protocol for these visits are indicated in Table 3.1.

Participants were assessed for local and systemic adverse events using a focused history and physical examination. Blood were also be taken for exploratory immunology analysis as detailed in Table 3.1.

3.2.8.1 Visit 3: Day 3 Follow-up

This follow-up visit took place at day 3 ± 1 day either side of the target date determined by the date of biting visit. The volunteers were assessed for local and systemic adverse events using a focused history, physical examination and routine haematology and biochemistry blood tests. A urine sample was taken for urinalysis from all volunteers. Blood were also taken for exploratory immunological analysis.

3.2.8.2 Visit 4: Day 10 Follow-up

This follow-up visit took place at day 10 ± 3 days either side of the target date determined by the date of biting visit.
The volunteers were assessed for local and systemic adverse events using a focused history, physical examination and routine haematology and biochemistry blood tests. A urine sample was taken for urinalysis from all volunteers. Blood were also be taken for exploratory immunology analysis.

3.2.8.3 Visit 5: Day 21 Follow-up

The final study follow-up visit took place at 21 ± 5 days. Blood were also be taken for exploratory immunology analysis.

3.2.8.4 Focus Groups

Two focus groups took take place with all the volunteers from the sand fly biting study after the final day 21 follow-up visit. The maximum number considered suitable for a focus group

is 12 (Caroline Tynan and Drayton, 1988). The focus group took place at the University of York, York, YO10 5DD.

The aim of the focus groups was to elicit the volunteer's experiences of participation in the study in greater depth and gather information to help inform the design of the subsequent CHIM study. The focus groups were digitally recorded (with consent) and each lasted around 3 hours, which was similar in time based upon the initial PPI activity (see section 2.3.1). The focus group activity was led by GJ, who acted as an independent chair, with input from myself and the rest of the clinical research team. The focus groups were subsequently fully transcribed verbatim and analysed using NVivo software (QSR International Pty Ltd). An inductive content analysis approach was be used (Elo et al., 2014). To establish the trustworthiness of the analysis, one member of the team independently read the transcripts line by line to identify emergent themes (Noble and Smith, 2015). A second member of the team independently check a proportion of these to verify the coding. Discussion of, and agreement upon, common patterns and broader themes from the volunteer's experiences was reached between both the independent health psychology team and the clinical team. Any dissident views and areas of diversity were considered and discussed with the wider study team.

Participants were be asked about their experiences of taking part in this study, centred on the following points:

(a) The recruitment process (to include screening and pre-screening)

(b) The procedures involved in the sand fly biting visit itself

(c) The experience of being bitten, and the subsequent evolution/healing of the bite

(d) The follow-up visits (to include testing procedures/photography)

(e) Any safety concerns and if so how the participants felt about the processes in place to mitigate these

(f) If taking part in this study is what the participants expected and if not, what was different or unanticipated

(g) Potential improvements for subsequent studies

(h) The facilitators will provide a description of the proposed infected sand fly biting study, and will ask the participants about any suggested improvements to the process or any anticipated barriers/facilitators to taking part

(i) The facilitators will provide a description of the various potential treatments for cutaneous leishmaniasis, and the participants will be asked for their views on acceptability/optimal choices

(j) Any other discussion points raised by participants

3.2.8.5 Additional Visits

Additional visits and assessments, if required, were to evaluate an adverse event and/or to identify a diagnosis. As per usual clinical standards of care, a biopsy might be necessary to provide a differential diagnosis of macular lesions, at some stage of the study, although this was not necessary during this study.

	Pre-screening Days -40 to -14	**Visit 1** Screening Days -30 to -7	**Visit 2** Sand fly biting Day 0	**Visit 3** FU Day 3	**Visit 4** FU Day 10	**Visit 5** FU Day 21
Window	N/A	N/A	N/A	± 1 day	± 3 days	± 5 days
Information & discussion with the volunteer	X	X				
Consent		X				
History, Examination		X	X	X	X	
FBC, U+E, LFT, CRP		X		X	X	X
IgE		X		X		X
Blood borne virus screen (HIV, Hepatitis B/C)		X				
Sand fly saliva antibody		X				X
Leishmania antibody		X				
Cellular response (sand fly proteins)		X		X	X	X

Serum β-HCG pregnancy test		X				
Sand fly biting & documentation			X			
Photography / Dermoscopy			X	X	X	X
Videography			X			
Urinary pregnancy test					X	X
Blood volume		20ml	0ml	15ml	15ml	20ml

Table 3.1: Schedule of visits

This table shows all testing and procedures conducted at each study visit. The focus group is excluded from this table and is discussed elsewhere.

3.2.9 Sand fly salivary gland IgG enzyme-linked immunosorbent assay (ELISA)

As an exploratory study, specific anti-saliva IgG for both *Phlebotomus papatasi* and *Phlebotomus duboscqi* was measured by enzyme-linked immunosorbent assay (ELISA) as per the below method. This methodology is well described but involves dissection of sand fly salivary glands to produce sand fly salivary gland antigen containing lysate (Rohousova et al., 2005; Sumova et al., 2018). This study reviewed the feasibility of using the methodology as part of any exclusion criteria for the future CHIM study. *Phlebotomus duboscqi* salivary gland lysate was used for the sera of those participants exposed to *Phlebotomus duboscqi,* and *Phlebotomus papatasi* salivary gland lysate was used for sera of those participants exposed to *Phlebotomus papatasi.*

1. Coat plate with salivary gland antigen lysate (SGL). One SGL is equivalent to one whole dissected sand fly salivary gland.
    - antigen at 1 salivary gland/ul, use 1/5 SGL per well
    - dilute in carbonate-bicarbonate buffer (pH 9)
    - add 100ul/well
    - incubate at 40°C overnight
2. Wash plate 2x PBS-Tw (PBS buffer with 0.05% Tween 20), 100ul/well
3. Block plate with 6% low fat milk powder diluted in PBS-Tw
    - 100ul/well
    - incubate at 37°C for 60min
4. Wash plate 3x PBS-Tw, 100ul/well
5. Serum samples are diluted in 2% low fat milk powder
    - use at 1:100 for samples tested with SGL
    - 100ul/well
    - samples run in triplicate
    - incubate 37°C for 90min
6. Wash plate 5x PBS-Tw, 100ul/well
7. Dilute anti-human IgG horseradish peroxidase (Sigma A6029) 1:1000 in PBS-Tw
    - 100ul/well
    - incubate 37oC for 45min

8. Wash plate 5x PBS-Tw, 100ul/well
9. Dilute OPD (o-Phenylenediamine) substrate tablets (Sigma P9187)
    - one OPD tablet + one buffer tablet in 20ml water
    - allow to dissolve, add 100ul/well
10. Incubate at room temperature in the dark for more than 5min
    - read plate every 5 minutes at 492nm
    - stop reaction when optical density reaches the cut-off value
11. Add 100ul/well 10% sulfuric acid solution
12. Read ELISA plate at 492nm

3.2.10 Assessment of safety

Safety was monitored both actively and passively during the study. On the day of sand fly biting the clinical investigator constantly examined the patient for risk of anaphylaxis during the sand fly biting procedure. Participants were encouraged to voice any issues during the intervention period. A pathway to act on potential anaphylaxis and other emergencies was developed to ensure safety and a standardised approach (see appendix Figure 7.15).

No clear guidance exists to determine the minimum standards of emergency resuscitation equipment for clinical studies conducted outside of hospital settings. Quality standards do exist for Primary Care settings where emergency care may be needed (Resuscitation Council UK, ,2023). Suggested equipment includes oxygen, oropharyngeal airways, self-inflating bag with reservoir airway devices, supraglottic airway devices, automated external defibrillator (AED), intravenous cannulae, infusion fluid (crystalloid), and adrenaline 1 mg (= 10 ml 1:10,000 for cardiac arrest) as prefilled syringes. In addition, it is recommended that algorithms and emergency drug doses are readily available, and staff skilled in the management of clinical emergencies and resuscitation (ALS/Advanced Life Support trained). The kit that I assembled at the Translational Research Facility at the University of York, included the above but also glyceryl trinitrate (GTN) spray, salbutamol aerosol inhaler and spacer device, amiodarone pre-filled syringes (for cardiac arrest) adrenaline injection (1:1000, 1mg/ml for anaphylaxis), aspirin dispersible tablet (300mg), glucagon injection (1mg), oral glucose gel, and buccal midazolam, given the potential emergencies scenarios

that may present (Simpson and Sheikh, 2010). There was also a significant stock of clinical consumables such as syringes, needles, gauze and dressings. Pulse oximetry, blood pressure machine and temperature probe were also present, as used for baseline observations. Due to the provision of tubed oxygen, which would be required in the case of an unwell participant, particularly if evidence of anaphylaxis and/or airway involvement, there remains a risk of automated defibrillation causing burns and fire if high-flow oxygen is present. As such and due to the clinical expertise of the study team, a semi-automatic defibrillator was used to ensure that defibrillation could be controlled to avoid any risks.

Solicited adverse events were determined prior to study initiation and included:

- Itch
- Pain / Discomfort
- Erythema (redness)
- Swelling
- Blister / Bullae
- Ulceration

A physical diary card was given to each participant to record subjective systemic and local solicited adverse events on a 0-10 visual analogue score. The diary card also allowed recording of unsolicited adverse events, any contact with healthcare professionals, and any additional new medications. Participants were provided with contact deals for an emergency contact line which was available 24 hours a day, that I manned. A further credit card-sized contact card was provided with two intentions: to allow the emergency contact details to be accessible, but also to alert other healthcare professionals to the study and access to clinical investigators if needed given the potential difficulty in explaining the nature of the study.

Only two participants underwent sand fly biting on any given day, and no participants underwent sand fly biting simultaneously. The development of any Serious Adverse Event (SAE) or grade 3 Adverse Event (AE) at day 3 post-biting considered possibly, probably or definitely related to the biting procedure will result in a temporary halt and review of the sand fly biting parameters (see definitions). Therefore, the safety outcomes were reviewed 3 days after all biting procedures in real time for all subjects. If an SAE or grade 3 AE had

been recorded the CI and PIs would review both the clinical event, and the biting parameters in terms of the number of sand flies, the species and length of exposure, with regard to progression of the study. If Grade 3 / exaggerated reactions had been observed it would be likely that the Investigators (as above) would seek a protocol amendment to decrease the number of sand flies present within the biting chamber, or length of exposure to mitigate any exaggerated response. At the discretion of the clinical investigators it would be possible that a sufficient number of grade 2 AEs, would also result in such a halt to the study and consideration of a protocol amendment. These adverse events were graded using a modified version of grading criteria developed by the National Institutes of Health (NIH) (U.S. Department of Health and Human Services, 2021).

Definitions

3.2.10.1 Adverse Event (AE)

An AE is any untoward medical occurrence in a volunteer who has consented and is participating in a clinical study, including occurrences which are not necessarily caused by or related to that investigational item (in this case Sand Flies). An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease occurring during such a clinical study.

Information on adverse events will be collected on the day of sand fly biting and at follow up visits as defined in the schedule. AEs will be recorded by study staff through direct questioning and examination. Events will be graded according to the table in Appendix 1. In addition, systemic laboratory adverse events will be collected through routine laboratory testing according to the schedule. These will be recorded on the standard laboratory report. The information obtained will be recorded in the volunteer's file.

3.2.10.2 Serious Adverse Event (SAE)

An SAE is an AE that results in any of the following outcomes, whether or not considered related to the sand fly bite.

- Death (i.e. results in death from any cause at any time)

- Life-threatening event (i.e. the volunteer was, in the view of a clinical investigator, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more serious form, might have caused death.
- Persistent or significant disability or incapacity (i.e. substantial disruption of one's ability to carry out normal life functions).
- Hospitalisation, regardless of length of stay, even if it is a precautionary measure for continued observation. Hospitalisation (including inpatient or outpatient hospitalization for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a serious AE.
- An important medical event (that may not cause death, be life threatening, or require hospitalisation) that may, based upon appropriate medical judgment, jeopardise the volunteer and/or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic reaction requiring intensive treatment in an emergency room or clinic, blood dyscrasias, or convulsions that do not result in inpatient hospitalization.
- Congenital anomaly or birth defect.

Site	Adverse Event
Local (at the site of bite)	**Symptoms** Itch Pain / Discomfort **Signs** Erythema (redness) Swelling Blister / Bullae Ulceration

Table 3.2: Solicited Adverse Events

See appendix Table 7.3 for further explanation of adverse events.

3.2.10.3 Causality Assessment

For every AE, an assessment of the relationship of the event to the sand fly bite was undertaken by a Clinical Investigator. An interpretation of the causal relationship of the intervention to the AE in question will be made using clinical judgment, based on the type of event; the relationship of the event to the time of sand fly bite and alternative causes such as intercurrent or underlying illness and concomitant therapies.

0	No Relationship	Adverse events that can be clearly explained by extraneous causes and for which there is no plausible association with study product. Or adverse events for which there is no temporal relationship
1	Unlikely	Adverse events that may be temporally linked but which are more likely to be due to other causes than this study
2	Possible	Adverse event that could equally well be explained by the study or other causes, which are usually temporarily linked. Or of a similar pattern of response to that seen with other vector biting studies.
3	Probable	Adverse events that are temporarily linked and for which the study product is the more likely explanation than other causes. Or known pattern of response seen with other vector biting studies.
4	Definite	Adverse events that are temporarily linked and for which the study product is the most likely explanation. Or known pattern of response seen with other vector biting studies.

Table 3.3: Causality assessment criteria for adverse events

3.2.10.4 Reporting Procedures for All Adverse Events

All adverse events occurring during the study observed by a Clinical Investigator or reported by the participant whether or not attributed to study procedure were recorded in the CRF. The severity of clinical and laboratory adverse events was assessed according to the AE grading. All adverse events that resulted in a participant's withdrawal from the study or that were present at the end of the study, will be followed up until a satisfactory resolution occurs, or until a non-study related causality is assigned.

3.2.10.5 Unexpected screening findings

If there were any abnormal or unexpected findings from these screenings, this will be discussed with the participant by the clinical team. The participant will be referred to an appropriate medical speciality, or their GP.

3.2.10.6 Reporting Procedures for Serious Adverse Events

The Investigator will complete an SAE report form and the Sponsor immediately (within 24 hours) of becoming aware of an SAE. All SAEs that are causally linked to the administration of the protocol and unexpected will be reported to the REC within 15 days of being made aware of the event. In addition, all grade 3 & 4 AEs regardless of unexpectedness will be referred to the REC in consideration that these would constitute a halt to the study and/or protocol amendment.

3.2.10.7 Withdrawal of Volunteers

A volunteer has the right to withdraw from the study at any time and for any reason and is not obliged to give his or her reasons for doing so (including during the Sand Fly biting itself). A Clinical Investigator may withdraw the volunteer at any time in the interests of the volunteer's health and well-being. If withdrawal is due to an adverse event, appropriate follow-up visits or medical care will be arranged until the adverse event has resolved or stabilised. If a volunteer is considered to have failed the screening assessment or withdraws from the study at any time, either by choice or on the recommendation of clinical

personnel, data and samples collected up to that point will remain available for analysis as part of the study.

If the volunteer is not enrolled into the biting portion of the study after being deemed ineligible, all screening data will be destroyed. If there is deemed to be any information relevant for the participant's GP to be made aware at this stage, consent will be taken to write to the GP.

If a volunteer who has withdrawn from the study requests for their existing, un-analysed samples to be destroyed or for their data to not be included in reports/ statistical analyses, the CI will take responsibility for ensuring that appropriate action is taken.

If there is sufficient time remaining, then volunteers will be replaced in the study if they withdraw. A standby list of volunteers will be maintained.

3.2.10.8 Pregnancy Reporting

If a female volunteer becomes pregnant during the 90 days after biting visit, she will be followed up as other volunteers and in addition will be followed until pregnancy outcome. The pregnancy will be reported to the Sponsor in accordance with reporting for research-related AEs

3.2.11 Clinical criteria for temporarily Interruption or discontinuation of the Study

Definitions:

"Discontinuation" is the permanent withholding of further sand fly exposure visits from all or some study groups in the study.

"Interruption of sand fly biting" is the temporary withholding of sand fly exposure if a serious adverse reaction is experienced during the study.

If any of the following occur, interruption or discontinuation of all further sand fly biting will take place and the Sponsor will be notified within 24 hours.

(1) Death in any subject in which the cause of death is judged to be possibly, probably or definitely related to sand fly bite exposure

(2) The occurrence in any subject of an anaphylactic reaction to sand fly bite exposure

(3) If 2 or more participants experience an unexplained, unexpected grade 3 or 4 clinical or laboratory event (confirmed on attendance or repeat testing) that has not resolved within 72 hours and considered possibly, probably or definitely related sand fly bite exposure.

The Sponsor will notify the Study Management Group (SMG) and the SMG and / or the Sponsor will determine whether or not to call an unscheduled meeting to review the safety data, and whether or not to hold further sand fly biting visits until this has taken place.

If the study is halted or stopped for a reason involving risk to a volunteer's health or safety then an Urgent Safety Measure will be implemented in accordance with the Safety Reporting SOP. If the study is halted or stopped for any other reason the CI or Sponsor representative will notify the HRA not later than 15 days from the date of the halting of the study in accordance with safety reporting practices. Restarting the study will be a substantial amendment.

3.2.12 Reimbursement for Volunteers

Volunteers will be compensated for their time and for the inconvenience at approximately these rates in relation to the following visits:

- Screening visit - £60,
- Sand fly biting visit - £100,
- Follow-up visits - £25 (3 expected)
- Focus group £60
- Total £295

Travel costs were reimbursed in relation to expenses submitted, in addition to the above.

Additional treatments and investigations were provided free of charge for volunteers who require it, including by formal referral to the appropriate NHS service.

3.2.13 Data Analysis

Comparison between vectors were made using independent t-test (continuous data), Mann Whitney (ordinal data) or chi-square tests (categorical data). Data tables are reported as mean (SD) or n (%). Statistical significance was demonstrated by a p-value of <0.05.
IBM SPSS Statistics for Windows (Version 26.0. Armonk, NY; IBM Corp) was used to conduct analyses. Summaries of adverse events reported in the study are presented as summed data for all participants per adverse event or summed VAS score for each participant across all adverse events. Data are presented as median and range. Graphs were generated in GraphPad Prism v9.0.1.

3.3 Results

3.3.1 Participant characteristics

55 participants were pre-screened and assessed for eligibility from October 2019 to November 2019. 24 participants attended for an in-person screening visit, and subsequently 12 participants were eligible for entry and were entered into the intervention phase of the study (Figure 3.3). 6 participants were entered into each arm, allocated to each vector, *P. papatasi* or *P. duboscqi.* The sequence of allocation wasbased on availability of the respective sand fly species and date of recruitment. The follow-up period lasted until January 2020 with participants completing study visits out to day 21 post-sand fly biting (+/- visit window). 100% (12/12) of participants completed the study visits as outlined. No additional visits were required, and none were lost to follow-up. All participants were invited to attend the focus group, 83.3% (10/12) were able to attend. Due to logistics and participant availability, two focus groups of five participants each were conducted. Two participants were unable to attend due to other unrelated commitments. These participants were invited to submit any additional comments and feedback via email but did not wish to take this up.

Of the 55 participants who expressed an initial interest and were pre-screened a total of 31 participants were not entered for formal screening. The 15/31 declined further involvement. 7 participants were excluded based on past medical history, and 2 participants

were excluded based on travel history that would suggest that they have had prior potential sand fly and/or *Leishmania* exposure risk. An exclusion based on travel history was important given the procedures were intended to largely mirror any formal Leishmania CHIM, where prior sand fly/*Leishmania* exposure could protect against lesion development. 50% of participants (12/24) were excluded from further participation after formal in-person screening. 9 participants were excluded based on elevated serum Total IgE, outside of the normal physiological range. Of these 9 participants, 3 had additional unexpected medical findings noted at screening, 2 of which were major contributors to exclusion. 2 participants were excluded solely on unexpected medical findings noted at screening. 1 participant was excluded based on travel history after direct questioning.

12 participants were entered into the biting study, with n=6 in each arm. Five female participants and one male participant were recruited to each arm. Sex was self-reported. Mean age in the *P. papatasi* sand fly arm was 40.8 years±12.8 years, compared to the 39.5±11.9 years in the *P. duboscqi* sand fly arm. One participant had a tattoo and one had a previous persistent scar from an insect bite. No other lesions or significant skin conditions were recorded. 100% of participants denied a propensity to scarring, history of anaphylaxis, history of urticaria and any history of psoriasis. A mild history of atopy (asthma, eczema, hay fever) was noted for some participants (see Table 3.4)

		Sand fly species			
		Phlebotomus duboscqi		*Phlebotomus papatasi*	
		n	%	n	%
Gender	Female	5	83%	5	83%
	Male	1	17%	1	17%
Eczema	No	4	67%	5	83%
	Yes	2	33%	1	17%
Asthma	No	6	100%	4	67%
	Yes	0	0%	2	33%
Urticaria	No	6	100%	6	100%
Psoriasis	No	6	100%	6	100%
Propensity to scarring	No	6	100%	6	100%
Allergy to any medications (including OTC items)	No	4	67%	4	67%
	Yes	2	33%	2	33%
Allergy to non-medications (including hay fever)	No	4	67%	4	67%
	Yes	2	33%	2	33%
History of anaphylaxis	No	6	100%	6	100%
Smoking history	Current	0	0%	1	17%
	Never	5	83%	3	50%
	Former	1	16.70%	2	33%
Travel outside the UK in the last 12 months	No	1	16.70%	2	33%
	Yes	5	83.30%	4	67%
Travel outside Europe in the last 12 months	No	6	100.00%	4	67%
	Yes	0	0.00%	2	33%

Table 3.4: Baseline participant characteristics

Summary of baseline characteristics recorded at screening visit by physical examination and clinical history. 6 participants in each arm (sand fly species).

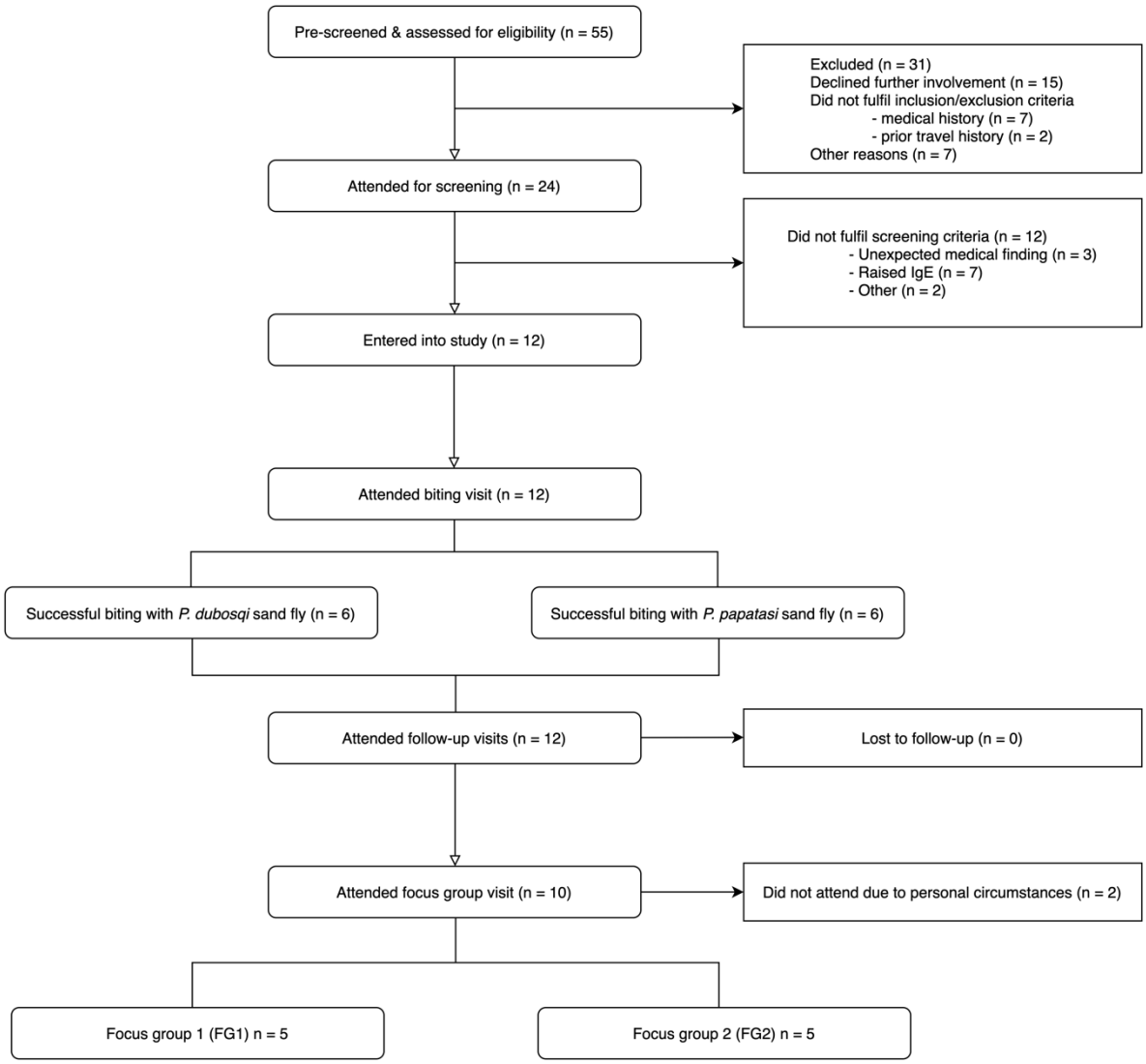

Figure 3.3: FLYBITE study flow diagram (CONSORT -Consolidated Standards of Reporting Trials)

Description of participant progress in the study including pre-screening, screening, study intervention.

Adapted from a figure produced for (Parkash et al., 2021a).

3.3.2 Primary outcome measures: Effectiveness of sand fly biting

All participants were exposed to 5 sand flies contained in a biting chamber, placed on the forearm for 30 minutes. 100% (12/12) of participants received at least one successful sand fly bite (see appendix Figure 7.16 and Figure 7.17, for images depicting progress of all participant's skin bite sites from biting until last follow-up). There was no noticeable difference between sand fly species in terms of mean number of sand fly bites (7.00±2.76 vs 6.33±5.39 for *P. papatasi* and *P. duboscqi,* respectively). Comparison of sand fly biting rate, defined as number of bites per 5 sand flies in 30 minutes also showed no significant difference (3.33±0.81 vs 3.00±1.26 bites for *P. papatasi* and *P. duboscqi,* p=0.56) although the study was not powered to detect a significant difference (see Table 3.6).

Successful biting was confirmed by one of two techniques. Visual inspection by the research team for evidence red translucent engorged sand fly abdomens after immediate removal of sand fly biting chamber, and then subsequent dissection of the sand fly abdomen to look for microscopic evidence of blood meal. There was no significant difference in the number of engorged sand flies post-biting between species upon dissection (3.33±0.82 vs 3.00±1.27 for *P. papatasi* and *P. duboscqi*, respectively) (Table 3.5). For many participants, bites remained visible at the final visit at day 21, although they had reduced in number (3.17±1.60 vs 3.50±3.73 for *P. papatasi* and *P. duboscqi*, respectively). This therefore demonstrates effectiveness of the model with 100% of participants receiving 1 sand fly bite, and in each case at least 1/5 sand flies demonstrated evidence of blood meal. All participants attended for each of their planned visits and attended within the window period as outlined at the onset. There were no additional visits and no visits were missed.

	Type						p-value
	Phlebotomus duboscqi			*Phlebotomus papatasi*			
	Mean	SD	n	Mean	SD	n	
30 mins -Reviewer 1: Number of bites	6	5	6	7	3	6	0.485
30 mins -Reviewer 1: Biting rate	1.3	1.1	6	1.4	0.6	6	0.485
30 mins -Reviewer 2: Number of bites	6	5	6	7	2	6	0.485
30 mins -Reviewer 2: Biting rate	1.3	1.1	6	1.4	0.5	6	0.485
90 mins -Reviewer 1: Number of bites	5	3	6	6	2	6	0.589
90 mins -Reviewer 1: Biting rate	1	0.6	6	1.2	0.5	6	0.589
90 mins -Reviewer 2: Number of bites	5	3	6	6	2	6	0.589
90 mins -Reviewer 2: Biting rate	1	0.6	6	1.2	0.5	6	0.589

Table 3.5: Comparison of sand fly biting between *Phlebotomus duboscqi* and *Phlebotomus papatasi*.

Total number of bites and biting rate (bites per 5 sand flies) are compared. Mean, standard deviation (SD) and p-values provided. The study was not powered to detect statistical significance. Adapted from a table produced for (Parkash et al., 2021a).

3.3.3 Primary outcome measures: Safety of sand fly biting

Solicited reactions have been discussed above, and are anticipated events based on the study intervention (graded using a modified version of grading systems by the National Institutes of Health (NIH) (U.S. Department of Health and Human Services, 2021). (see appendix Table 7.3). No grade 3 or higher adverse events were reported. Four grade 2 adverse events were noted, 50% (2/4) were unrelated - a new cardiac murmur at screening, prior to sand fly exposure (n=1) and a further participant (n=1) had a new cough at day 21, which was clinically consistent with a viral upper respiratory tract infection and assigned as unrelated. Two study-related grade 2 adverse events were noted, both were solicited: erythema at the sand fly bite site and persistent itch at the sand fly bite site. There were no unsolicited causally study-related grade 2 or greater adverse events.

No suspected unexpected serious adverse reactions (SUSARs) were reported during the study. Little change was observed in IgE (KU/L) at baseline and during follow-up for *P.papatasi* (24.53±13.88) vs *P.duboscqi* (38.87±28.13). Mean CRP (normal range <5mg/L) for *P. papatasi* and *P. duboscqi* on day 3 post-biting (5.00±4.24 vs 1.67±1.15), day 10 post-biting (3.67±2.31 vs 7.67±10.69) and day 21 post-biting (5.00±1.73 vs 2.00±1.73) was not influenced by vector species. These findings confirm safety of uninfected sand fly biting on healthy volunteers.

		Mean *P.papatasi*	Standard deviation *P.papatasi*	Mean *P.duboscqi*	Standard deviation *P.duboscqi*
Baseline bloods	Total white cell count (x 10^9/L)	6.78	1.61	6.55	2.06
	Eosinophils (x 109/L)	0.13	0.08	0.10	0.09
	C-reactive protein (mg/L)	2.67	2.08	1.67	1.15
	IgE (KU/L)	24.53	13.88	38.87	28.13
Biting Day (Day 0)	Flies fed	3.33	0.82	3.00	1.26
	Bites visible (30 minutes)	7.00	2.76	6.33	5.39
	Bites visible (90 minutes)	6.17	2.40	5.00	2.97
Day 3 post-biting	Bites visible	2.67	0.82	3.00	2.97
	Size of biggest lesion (mm)	3.67	2.88	1.00	0.89
	Total white cell count (x 10^9/L)	7.25	2.45	6.38	1.61
	Eosinophils (x 109/L)	0.12	0.08	0.13	0.10
	C-reactive protein (mg/L)	5.00	4.24	1.67	1.15
	IgE (KU/L)	22.70	13.44	32.80	23.39
Day 10 post-biting	Bites visible	2.67	1.37	2.83	3.37
	Size of biggest lesion (mm)	3.50	3.56	3.00	4.69
	Pain (0-10)	0.00	0.00	0.00	0.00
	Itch (0-10)	0.00	0.00	1.00	2.45
	Erythema	1.00	0.63	0.83	0.98
	Swelling	0.17	0.41	0.33	0.52
	Blistering	0.17	0.41	0.17	0.41
	Total white cell count (x 10^9/L)	7.88	1.43	5.98	1.23
	Eosinophils (x 109/L)	0.15	0.10	0.17	0.12
	C-reactive protein (mg/L)	3.67	2.31	7.67	10.69
Day 21 post-biting	Bites visible	3.17	1.60	3.50	3.73
	Size of biggest lesion (mm)	2.50	1.38	2.33	2.42
	Erythema	1.17	0.75	1.00	0.89
	Swelling	0.33	0.52	0.33	0.52
	Blistering	0.00	0.00	0.00	0.00
	Pain (0-10)	0.00	0.00	0.00	0.00
	Itch (0-10)	0.00	0.00	0.17	0.41
	Total white cell count (x 10^9/L)	7.47	2.37	6.48	0.69
	Eosinophils (x 109/L)	0.10	0.09	0.17	0.14
	C-reactive protein (mg/L)	5.00	1.73	2.00	1.73
	IgE (KU/L)	22.77	12.99	33.13	22.69

Table 3.6: Summary of biting data

Adapted from a table produced for (Parkash et al., 2021a).

3.3.4 Secondary outcome measures: Visual analogue scores

The participant experience was captured with two methods both quantitative (visual analogue daily diary card) and qualitative (post-sand fly focus group). A diary card of daily symptoms out to the final day 21 visit (+/- visit window period) was recorded and included daily symptoms from 90 minutes following cessation of sand fly biting. This was used to capture subjective participant symptom data between study visits. A 100% response rate for each day was recorded for all participants. A visual analogue scale (VAS) (Adam et al., 2012) on a 10-point scale was used by participants to record their daily subjective experience for each of itch, pain/discomfort, erythema, swelling and blistering at the bite site; and systemic effects such as headache, malaise, myalgia and fever (see Figure 3.4 & Figure 3.5). The mean VAS scoring was between 0-1 for each measure out to day 21. The most frequently reported solicited effects were erythema, swelling and itch at the bite site (see Figure 3.4). A small number of participants attended for their final visit after day 21 (due to the window period and availability) and were allowed to record diary card data until this visit. The mean score for erythema and swelling for these days following day 21 was between 2-3, although there were only limited participant responses on these days, as most participants had completed their involvement in the study by this point. The subjective diary card data reinforced that the sand fly biting was well-tolerated with minimal adverse effects.

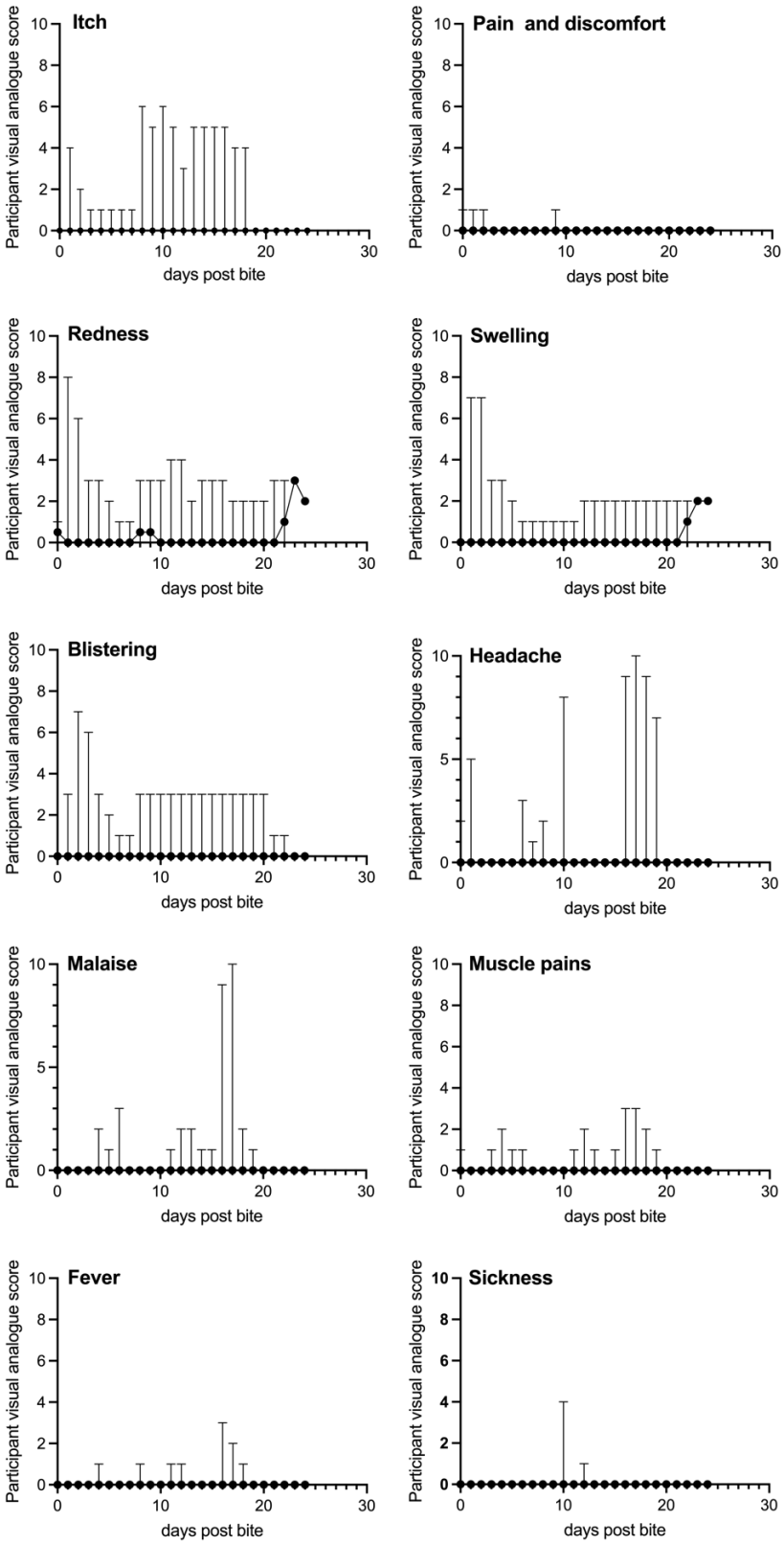

Figure 3.4: Visual analogue scores by type of adverse event

Summed VAS for all participants for each day following sand fly biting. Circles indicate median, range indicated by vertical bar. Adapted from a figure produced for (Parkash et al., 2021a).

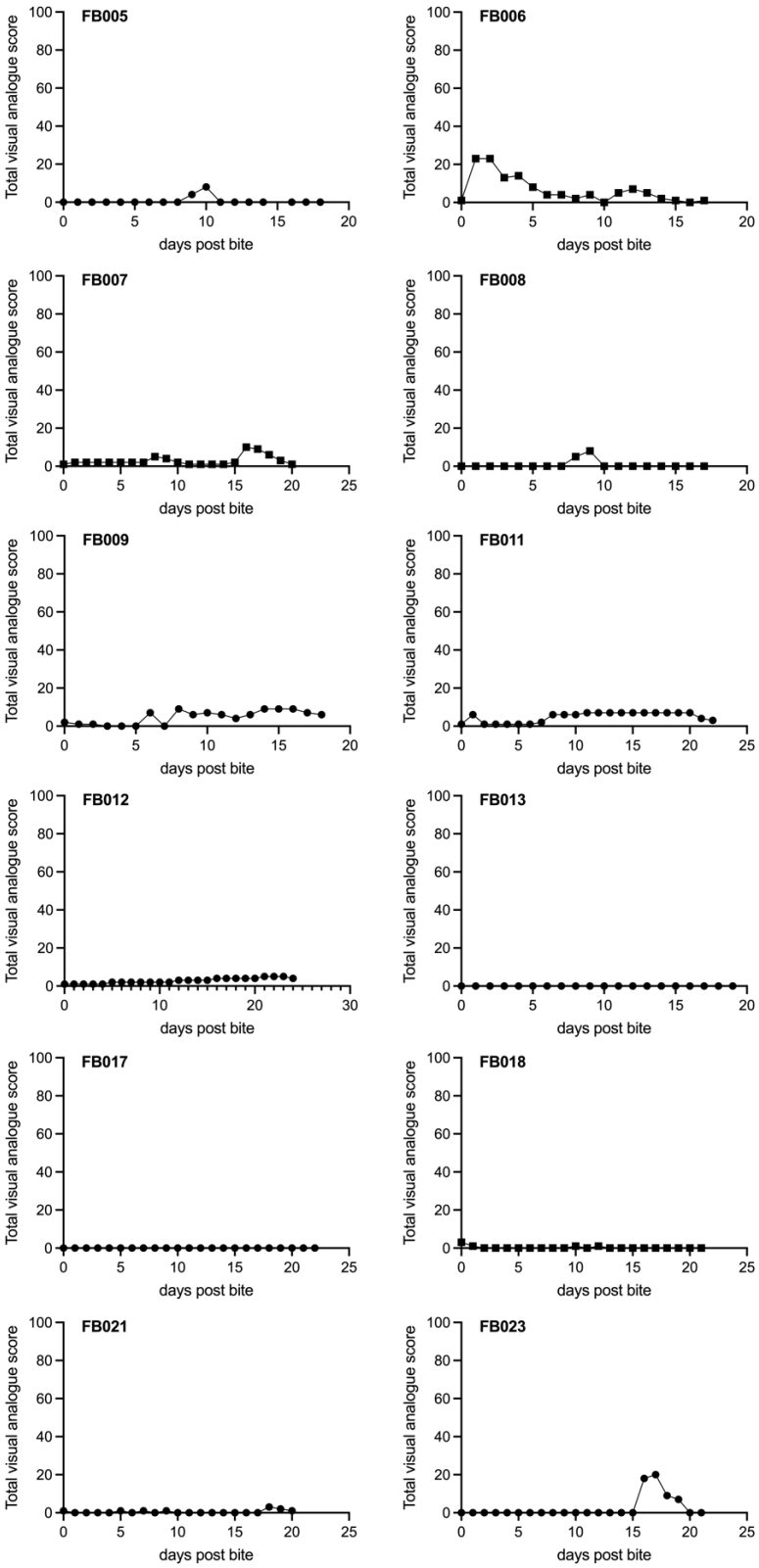

Figure 3.5: Total visual analogue scores for each participant

Summed VAS of all adverse events for each participant for each day following sand fly biting. Circles indicate median, range indicated by vertical bar. Adapted from a figure produced for (Parkash et al., 2021a).

3.3.5 Sand fly salivary gland IgG enzyme-linked immunosorbent assay (ELISA)

The ELISA results show limited evidence of stimulation of specific anti-saliva IgG from sera of participants exposed to either *Phlebotomus papatasi* or *Phlebotomus papatasi*. There was a wide variation in responses and only 1/12 participants had a statistically significant increase in IgG following sand fly biting.

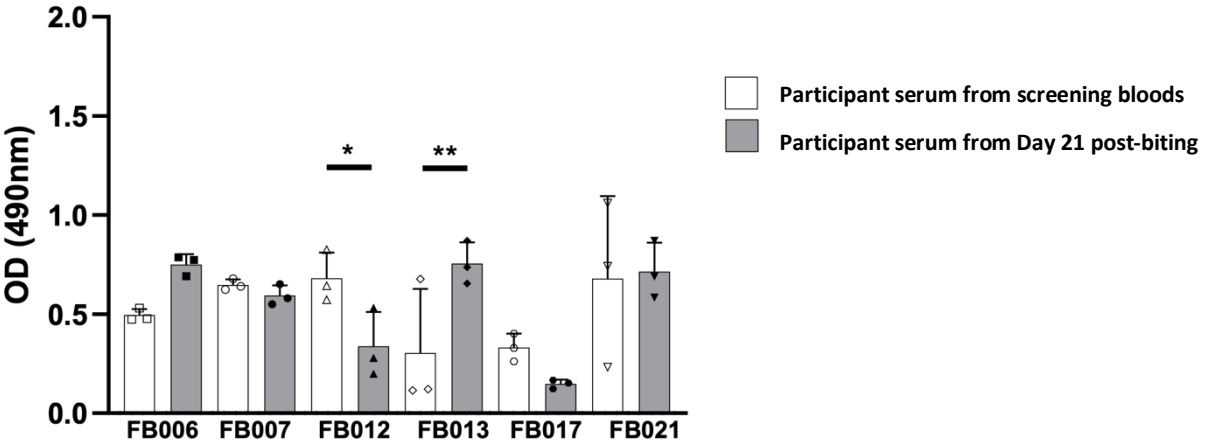

Figure 3.6 Anti-salivary gland IgG ELISA for Phlebotomus papatasi

Graph depicting pre- and post-uninfected sand fly challenge response to salivary gland proteins on naïve human volunteers. Solid bars represent mean, error bars represent standard deviation.

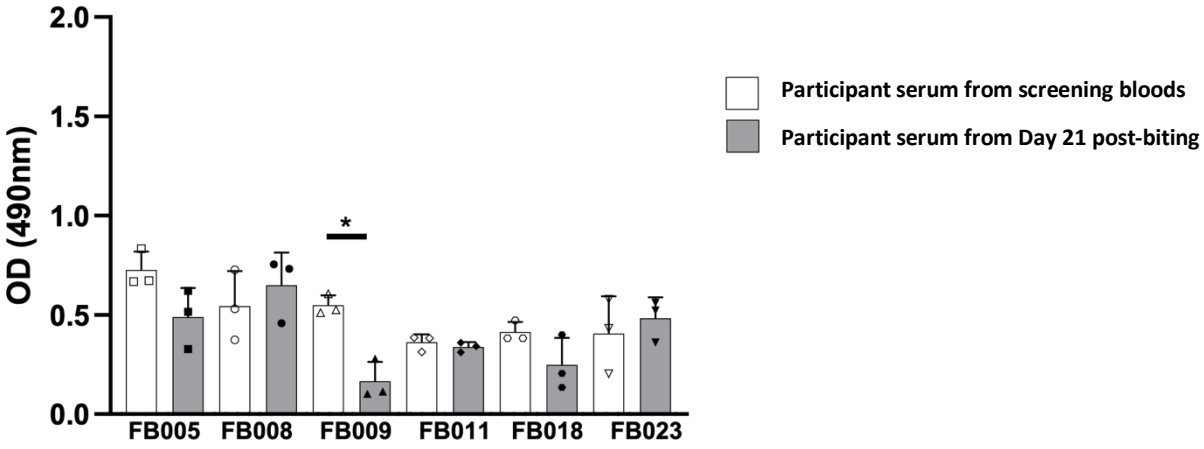

Figure 3.7: Anti-salivary gland IgG ELISA for Phlebotomus duboscqi

Graph depicting pre- and post-uninfected sand fly challenge human response to salivary gland proteins on naïve human volunteers. Solid bars represent mean, error bars represent standard deviation.

3.3.6 Focus groups

In total, 10 participants took part in two focus groups (FG1 and FG2). Each focus group consisted of five participants (each had four female and one male participant). The participants were assigned to each group based on availability and there was a mix of participants from each arm in both focus groups. In FG1, the age range of the female participants was 26 -51 years; in FG2, the age range of female participants was 21-59 years. The male participants were aged 38 and 40 years old, respectively.

Six overarching themes were identified:

i) Recruitment,

ii) Quality of the participant-facing information,

iii) Arrival and booking in process,

iv) Screening process,

v) Experience of being bitten,

vi) Overall study experience.

These themes are also described in detail with the sub-themes and selected quotes in appendix Table 7.4. The homogeneity in themes across the two focus groups is demonstrated by a coded comparison diagram
Figure 7.18.

i) Recruitment

The participants were informed of the study via several recruitment methods including local and national publications, posters, newsletters and mailing lists:

"I found out [about the trial] through the staff bulletin 'cos I read that when it comes through on Thursday at work and I saw it in there and it sounded interesting." (P3, FG1)

"I spotted the poster thinking back and thought that looks interesting and then the process was much the same after that but yeah I saw the poster and that's how I got involved." (P6, FG1)

"I actually saw the advertisement in the Local Link which is a free magazine that people in the area receive." (P16, FG2)

Some participants voiced their approval about the content of the posters, in getting a sense of the methodology:

"what appealed to me about the poster was that you actually showed the biting chamber because I think that was really useful because otherwise I would have thought that I'd have perhaps been in a room being bitten by flies" (P10, FG1)

There were many suggested points for improvement with respect to the poster and advertisements:

"there was nothing particularly about it that, other than the fact that I knew what it was about. Had I not been interested or had ever done scientific research before I probably wouldn't have noticed it" (P15, FG2)

"It looked exactly like a job advert." (P15, FG2)

"P2: I was interested to know if you'd linked up that there was an article and the advert.

P16: No.

P15: I don't think so. But I suppose that's fine because some people that read the whole local link would have read the article and then looked for the ad and then some people wouldn't have done and seen the ad anyway." (FG2)

"had I not been sat in front of it [study poster] was probably not as eye catching as it might have been." (P6, FG1)

"it definitely could have been more eye catching. If you wanted more people to respond and it need to have more prominence then yeah you could have done with a bigger word, a picture you know, something, a slogan" (P15, FG2)

"it [the study poster] didn't really sell what you wanted to do because the research it wants to help people all over the world that are affected with this fly. I think if they'd have focussed on actually people that want to save the world and help out it might have got more people in." (P14, FG2)

The study website was not widely accessed, although described as clear and informative:

"it [the study website] was good, it was clear. I can't remember a lot about it other than the image and that it gave me the information I needed." (P3, FG1)

Some participants expressed they had no need to access the website due to assimilating information from direct contact with investigators:

"I didn't think it was necessary. There was enough communication with Viv and everyone else to not need to use the website again." (P6, FG1)

The content of the website was also analysed, and suggestions about making a more personal story about peoples affected:

"I would be interested to know what's on it now. Does it have examples of other studies going on you know sort of globally Leish challenge or are there stories on it that tells you about people that are working in the countries with the victims or you know it would be interesting to know whether it, how much of a story it tells." (P15, FG2)

A range of alternative recruitment methods were also suggested to stimulate engagement, including social media and visual methods:

"maybe you should do like a Facebook ad, I don't know, something on social media." (P15, FG2)

"doing a little promotion video about the issue." (P15, FG2)

"you could do on Minster Fm or Radio York would probably do an interview someone about it if you tell them about it." (P15, FG2)

"local flyers and posters" (P13, FG2)

Presentations and talks were suggested as more persuasive means of driving engagement and accounting for the overwhelming nature of information fatigue that can be associated with detailed topics:

"some people are more easily persuaded by verbal means than written because we do live in a time of like too much information syndrome […] when everyone's presenting you with so much information I think on some level you just dismiss all of it." (P7, FG1)

Word-of-mouth was also reported to have played an important role in recruitment.

"somebody had told my wife and my wife told me and me and my wife was going for it. Unfortunately she wasn't successful due to health reasons but then I've been on the, I've been on it so it was all word of mouth." (P13, FG2)

Participants referred to the importance of publicising the remuneration for taking part. They stopped short suggesting advertising the full financial figure but suggesting the availability of financial compensation for taking part within the text of the advertisement was encouraged.

"it's important to say you on the poster that you will be compensated for the time you spend, I don't think there's a need to put a number on it." (P5, FG1)

ii) Quality of the patient-facing information

Participants suggested the written material appropriately specified the methods and details of the project:

"I think the [study information] sheets were completely adequate." (P15, FG2)

Some participants felt there was a disconnect with the level of detailed medical examination and would have appreciated more forewarning:

"P10: it may well have been I didn't read it closely enough. That could be quite-

P7: Yeah Viv did say to me.

P10: I knew I was going to have a medical.

P7: A full medical.

P6: No I agree I didn't appreciate it would be quite as extensive."

Although overall however, participants valued the thorough medical which served a secondary purpose as a health screening:

"I thought it was quite nice. I know I'm healthy. I just didn't realise that was going to be as extensive as it was." (P6, FG1)

It must be noted that some individuals had unexpected medical findings during the screening which did not preclude their involvement, but they were able seek medical attention and were grateful that these issues had been picked up. These quotes have been recorded in following sections.

Participants suggested a greater focus on the humanitarian nature of the project and perceived end-goals should be made:

"it [the future study] will take a lot more information to process in terms of I don't know what are the symptoms of the disease, what are the treatments, the potential sequels of either of those so it's a, I would think it's quite different" (P5, FG1)

iii) Arrival process

Participants were generally content with the booking in and administrative processes of the study, although did mention a few points to consider in future:

"a couple of times I arrived early at the lab bit and I'd cycled and it wasn't very nice weather but I had to kind of stand outside and wait until the exact time to be admitted into the building." (P16, FG2)

"[you could] let people know that you can just go round the corner to the main entrance of Biology and there's a lovely big lounge area that you can sit in" (P15, FG2)

iv) Screening process

The participants were reassured by the screening process which allowed them to better understand the nature of the project and to undergo a health screen. Indeed some participants had unexpected medical findings which did not preclude participation, but allowed for further medical follow-up:

"I had the suspicion of a hernia but I was sort of putting it off until I told Dr Viv about it and he investigated and said yeah, it is a hernia and you need to get it sorted properly so yeah it was very beneficial." (P13, FG2)

"we were treated very well especially as I say when the notice about the high blood pressure and Dr Viv was like you need to get it checked throughout the visit he was quite, he seemed genuinely concerned about it which was nice, he wasn't just treating you like a number he was treating like a person and taking care of you." (P13, FG2)

Some aspects of the screening process were not enjoyed by participants, including venepuncture, which was perceived to be more uncomfortable than the sand fly biting itself:

"the first time I had blood taken they couldn't get any blood out of one arm and so they had to do the other and that arm got quite bruised and was quite sore and it was quite, there was quite a lot of discomfort. The other subsequent visits were fine and it was very painless" (P3, FG1)

"I didn't like being weighed." (P3, FG1)

"it's vanishingly unlikely I could be pregnant there's still a certain anxiety associated with having a pregnancy test to me." (P10, FG1)

"it takes almost no time [to take height measurement] but it's like it's everyone's time writing it down, it's a section on the form I mean it's just a waste of resources. But then most of them make sense I mean they are taking I mean I don't know the blood test makes sense, the temperature, the weight, other stuff makes sense I think only one is a bit kind of weird is the height."

Participants voiced that the screening process had allowed the participants to build a relationship with the team, and the continuity of care of the clinical team strengthened these positive relationships. For some, the medical examination turned was more thorough than anticipated, although they were pleased overall to have undergone this:

"I thought it was quite nice. I know I'm healthy. I just didn't realise that was going to be as extensive as it was." (P6, FG1)

v) Experience of being bitten

The participants' experiences of the day of sand fly biting were largely positive:

"It was just a really nice experience. For me I mean, you know, I just found it all mod cons and everything was very comfortably ran because you just sit in a chair and back and yeah it was really nice, you know for my needs it was above and beyond what I needed." (P7, FG1)

Some anxiety was noted prior to the biting intervention:

"I was slightly anxious that morning because I thought, I know it's only a small area but I was thinking if they really really bite, I mean I come up quite badly with mosquito bites" (P10, FG1)

The sand flies were smaller than anticipated and the biting chamber was deemed to be relatively innocuous:

P14: "They were quite small yeah. I was expecting a bit bigger. (FG2)

P15: Because you couldn't really see them." (FG2)

The sand fly biting and main intervention were well tolerated, with some taking interest in the visualised sand fly engorgement as a result of the clear biting chamber:

"Slightly curious, really just like ok let's do science here. Strap it on and off we go […] You've been bitten by and insect before, you're going to volunteer to be bitten by seven now, let's see what it does." (P7, FG1)

"I was expecting more flies in there than just five. Not knowing what was going to be happening but I just expected more bites." (P13, FG2)

"Really excited when they started biting." (P10, FG1)

"It [the bite] was unremarkable to the point where I forgot I was taking part in a medical study." (P7, FG1)

The localised cutaneous effects of the sand fly bite were perceived to be minimal, and the skin reaction generally had been less than had been expected.

"[The bite itself] was really minor, much less, I mean just a tiny red mark and I was expecting you know a kind of a horrible itchy lump so it was much less than I expected." (P12, FG1)

"I only got bitten properly once and I could just feel the slightest sort of prickling, nothing painful." (P10, FG1)

Some participants had experienced itch, and one participant required further oral antihistamines which were provided by the study team:

"[The bite] was just itchy so the more I itched it and then it got the skin slightly torn and then just looked like an insect bite, just a scratched insect bite that I would itch which made it worse." (P15, FG2)

"I did get the antihistamines on my final visit just so that I could take some when it was itchy." (P15, FG2)

Although no other medication was required by any of the other participants:

"Just didn't need to [take antihistamines]" (P13, FG2)

Another participant suggested the presence of localised reaction that was visible was a reassuring feature of the study:

"I was happy in a way that I did have a slight reaction that something was happening." (P13, FG2)

The time commitment was questioned by one participant, despite being informed beforehand:

"the actual bite itself was about three hours in total with the half an hour of biting and then all of the monitoring stuff which did take out the afternoon." (P6, FG1)

The environment was felt to be very clinical and a little uncomfortable during the waiting period post-bite:

"You could have had a slightly more comfy chair to sit in. […] Because I was sort of sideways to the desk and you were stretching your arm out for quite a long time." (P10, FG1)

"[After the bite procedure] I thought you'd then get to sit in like a lounge area rather than the same clinical room, yeah. It didn't matter at all but that's just what I had in my head. That those three hours would be a little bit more comfortable in a way." (P15, FG2)

"I think the option perhaps for somebody to watch a film or, you know and then just be monitored in between, might be helpful for some people." (P10, FG1)

Some participants suggested that a phone app or text reminders on their phone might be useful to help complete the diary card:

"if you really wanted to bother like an app for you to quickly fill in your diary every day would have been amazing." (P15, FG2)

Some participants suggested preserving the option of a paper diary card would be useful in future:

"Well I work better with paper. I'm still a dinosaur when it comes to all the sort of technology and everything so for me it was fine to have to you know didn't forget and took it with me and everything so yeah." (P16, FG2)

With the exception of, the remaining participants felt confident they had received all the information they needed to get in touch with the study team.

One participant who struggled to remember the advice they were given in terms of follow-up and aftercare:

"I can't remember what the advice was." (P15, FG2)

Although the majority were comfortable with the processes during and after the study:

"If I'd needed to get help or something I felt quite confident that I would have been able to." (P17, FG2)

They also described positively the follow-up support networks that the team had put in place.

"it seemed that everything was in place fine." (P13, FG2)

vi) Overall study experience

The participants described their experiences positively and felt well-informed during the process. They singled out the team as professional and providing good communication.

P7: "it [involvement with the study and study team] was the most professional thing that's happened to me in a long time." (FG1)

"it was sort of a privilege to be a part of but like you say Viv had explained everything so well from what I say paperwork I never visited the website but anyway it was so clear there was not one point in the entire process for me was there any surprises it was exactly as sold and you know you just did it and it was fine, no surprises, no downsides, for me I mean it was a positive pleasant experience. I didn't, no apprehension at any point, there was no worry I mean I suppose I was just so well informed, due credit to them really." (P7, FG1)

The participants developed a good rapport with the study team, with one participant describing the team as *'warm'*.

"I'd applied for scientific research before but it wasn't as like, as warm as this one." (P14, FG2)

"you build up a bit of a relationship [with the team] don't you, you get to know them, have these chats with them and it was really pleasant. They were waiting for me every time I arrived there." (P3, FG1)

There were no safety concerns voiced by participants:

Investigator: "Did you have any safety concerns at all when you were part of the project? The trial.

P(Several): No. (FG1)

Investigator: None at all?

P(Several): No." (FG1)

The participants had opportunities to ask questions,

"we'd plenty of opportunity to ask questions to the people we were dealing with" (P10, FG1)

One participant felt that her commitment and time was valued:

"It was one of the nicest things about it that you felt that you were all involved in something interesting that they were genuinely excited about and that they appreciated your time and volunteering so that was really nice." (P10, FG1)

For some, the time commitment was more than they had anticipated and something to be considered in future:

"for the three hours I took a half day holiday and for this I'm trading time in lieu that I worked over Christmas so that's a commitment that I've had to make, which is fine but time commitment is an issue for most people I think." (P10, FG1)

There was a suggestion to use other virtual or electronic follow-up methods, although in-person contact was important and would wish this to continue:

"if it [taking bite photos] was beneficial to the study then I wouldn't mind doing it." (P13, FG2)

"you realise that whatever was going on was perfectly normal but you know had there been some sort of reaction or whatever you would like to think that you were being seen and they would pick something up or whatever it was because if you hadn't shown the bite you might think it was perfectly normal for it do something." (P17, FG2)

Most of the participants thought that the remuneration for taking part was appropriate for the time commitment involved:

it [the level of remuneration] was about right for the time commitment and for the amount of blood." (P10, FG1)

"I'd have done it for a lot less. I didn't, although I did it primarily for money I suppose I would have been perfectly happy well with sort of twenty quid…" (P10, FG1)

There were mixed motivations for taking part with the financial aspect being a deciding factor for some:

"it was a very interesting study but I think the main reason was I thought, ooh here's a study I can get paid for and raise some money." (P10, FG1)

"getting some compensation makes you feel like your time is valuable, you know I have very little free time and to do it for nothing and take a lot of time off I would be reluctant to do that […] I don't think everyone should have to do it for free, if you'd like to it's not a bad thing to get a bit of money as part of it." (P3, FG1)

Others ascribed less importance to remuneration, and expressed genuine altruistic interest in the research, a belief that the study was meaningful and worthwhile, and a desire to help others in the advancement of global health.

"it [the remuneration] wasn't relevant, I actually waivered my compensation so I don't. I mean I checked it the time commitment was doable as I work on campus and I have flexible hours I could work around it and ok it looks like I can do it so I went for it, I didn't really consider it." (P5, FG1)

"It was just nice to have done something maybe a bit meaningful for a change…" (P7, FG1)

"it's my chance to sort of contribute to some sort of research which in the long run is going to hopefully benefit quite a few people." (P16, FG2)

"I think it [the study] needs to be promoted more for what the end goal is rather than the actual process of it cos then I think more people like myself would be intrigued because they want to help things, they enjoy research, they enjoy helping things" (P14, FG2)

3.4 Discussion

Safety and effectiveness of sand fly biting was confirmed, which was determined by use of biting frequency and adverse effects data resulting from exposure of 12 healthy human volunteers to bites of either *Phlebotomus papatasi* or *P. duboscqi* the two natural vectors of *Leishmania major*. For all participants there was demonstrable evidence of at least one successful sand fly bite, and at least one sand fly that showed evidence of a successful blood

meal per participant. These results validate the documented protocol for achieving successful sand fly bites in humans that is safe and well-tolerated for participants. This is further reinforced by positive participant-reported experiences of being bitten by sand flies in a post-study focus group.

Sand fly biting studies have a long history with respect to animal models. There are some limited, experimental human exposure case studies. Non-infected sand fly bite has been studied to attempt transmission to sand flies from a cutaneous lesion, xenodiagnosis (Adler and Theodor, 1927b). Other studies have sought to demonstrate the sand fly bite reaction on humans, including the effects of recurrent sand fly bites, with implications on immunity to further *Leishmania* exposure (Oliveira et al., 2013)؛(Theodor, 1935). Several studies have subsequently shown transmission of *Leishmania* to human subjects using phlebotomine sand flies (Adler and Ber, 1941)؛(Swaminath, Shortt and Anderson, 2006)؛(Lainson and Strangways-Dixon, 1963). The results here are in keeping with the known biting studies described.

This is the first known study to directly compare biting rates on human subjects between the natural sand fly vectors of *Leishmania major, P. papatasi* and *P. duboscqi*. Both vectors have a similar mode of feeding. There have been no reported cases of serious adverse events such as anaphylaxis from exposure to these and other phlebotomine sand fly species, although severe reactions including anaphylaxis have been reported rarely in some other biting and hematophagous insects (Theodor, 1935)؛(Belkaid et al., 2000)؛(Klotz, Klotz and Pinnas, 2009). This reinforces the importance of demonstrating safety to ensure public confidence in future studies. This is also pertinent given many non-phlebotomine species of biting insects are colloquially known as ‘sand flies’ in different geographical regions, and these biting insects have been shown to demonstrate evidence of anaphylaxis in exposed humans.

The anti-sand fly salivary gland IgG ELISA did not demonstrate usefulness in determining serostatus in those participants naïve to sand fly saliva. As determined by clinical history taking, no participants had significant travel to regions where sand flies were present (defined as any history of travel within the preceding 30 days or history of more than 30

contiguous days stay in regions where Leishmania major transmitting sand flies are endemic). The majority of participants had travelled only within Europe during their lifetime.

There was wide variation in the IgG response with lack of consistent relationship between the cohorts of pre- and post- exposed sera. This was therefore not a useful to test to use for future sand fly biting studies within the human infection model. It has been noted that individuals with leishmaniasis exhibit a higher anti-salivary gland antibody response compared to healthy volunteers residing in endemic regions for sand flies. Therefore, healthy volunteer sera following the *Leishmania* CHIM could be tested for specific anti-salivary gland IgG retrospectively, but this ELISA is unlikely to be of benefit for prospective screening of candidate volunteers. Future work may also centre on repeating this ELISA using homologous antigen in parallel to also demonstrating specificity using heterologous antigen representing alternate species of sand fly. It is also noted that the dissection of sand fly salivary glands to produce sand fly salivary gland lysate is onerous. The use of anti-salivary gland IgG ELISA should therefore only be used in any screening after other inclusion/exclusion criteria have been satisfied if used.

Public and participant/patient engagement is now well embedded in research and has been associated with high quality research outcomes and improved research practices and accountability(Crocker et al., 2018),(Bagley et al., 2016). Prior to undertaking this study we carried out a public involvement (PI) exercise to inform the design and practical considerations in this research area (see chapter 2). This focus group exercise will inform future studies and improve our understanding of public perceptions of this type of research, with particular relevance to areas for improvement and barriers to involvement in a future CHIM study for sand fly-initiated cutaneous leishmaniasis. These results are in keeping with those reported by others in arthropod-delivered CHIM studies, where the anticipated harm is perceived to fluctuate over time. It was reinforced here that education is important to overcoming barriers to volunteering and negative connotations, in common with themes described elsewhere (Jao et al., 2020).

The complex and controversial history of deliberate experimental human infection is an important consideration within PI and focus group activities with contemporary CHIM-related studies (Darton et al., 2015). There is growing evidence of PI activity with other vector-borne CHIM studies, namely malaria (Jao et al., 2020), (Njue et al., 2018b). As new CHIMs develop, it is important to consider the robust frameworks around these studies, but also the role for PI and effective feedback and qualitive research to ensure robust practices with demonstrable accountability. With CHIM's also being considered in endemic regions, which may be relevant to future *Leishmania* studies, there is an ongoing debate about the ethics of studies in such circumstances and the need for engagement within a rapidly changing research landscape (Shah et al., 2020), (Gbesemete et al., 2020). These findings from our focus group demonstrate acceptability of sand fly biting on human participants. As a comparator, routine blood tests were seen to be less tolerable than the sand fly biting process itself.

Given the biological nature of the model, and the necessary limits to human experimentation as opposed to animal models, the number of bites sustained by each participant was unpredictable and not amenable to control other than by limiting the number of sand flies to 5, and controlling the time allowed for contact with sand flies. In most cases, the number of bites sustained by participants (as observed using dermoscopy immediately after the removal of the biting chamber) was in the majority of cases, higher than the number of sand flies that fed (recorded by noting macroscopic sand fly engorgement, and also microscopically by sand fly abdominal dissection for blood meal). This is explainable by the phenomena of sand fly probing, which is the observation that the sand fly investigates the skin with its proboscis, in order to identify blood vessels that are suitable for a prolonged feed, or to create a pool of blood from smaller vessels prior to settling for a sustained feed (Killick-Kendrick, 1999). During this probing time, no blood meal is taken, however infected sand flies emit saliva which can contain *Leishmania* parasites (Probst et al., 2001; Killick-Kendrick, 1999). The implication being that this behaviour improves efficiency of infection, and sand flies can infect the host even when a blood meal is not taken. This was first evidenced by experiments on monkeys bitten by sand flies, where *Leishmania* lesions subsequently developed but no observable blood cells were detected upon sand fly dissection (Probst et al., 2001). Sand fly feeding behaviour is also altered by

Leishmania infection which is an evolutionarily advantage. This has been noted in sand flies observed to have increased probing and biting tendency when infected with *Leishmania* (Probst et al., 2001; Rogers and Bates, 2007; Killick-Kendrick, 1999).

There are many well described and validated scoring systems for patient use in dermatological conditions. The visual analogue scoring system (VAS) is frequently used and has been shown to be valid and reliable for assessing dermatological manifestations in comparison with other scoring mechanisms (Adam et al., 2012), (Li, Liu and Herr, 2007). There is also evidence of correlation between quality-of-life scoring systems and VAS used in skin disease (Flytström et al., 2012). Although described in other arthropod biting studies (Reunala et al., 1997) the use of VAS here is the first known description of a dermatological scoring system used in a sand fly biting study. This strengthens its ongoing use with sand fly biting in future studies and is suggestive of potential use in other arthropod biting studies, including for CHIMs.

This study had some limitations. It is known that sand fly behaviour is altered following infection with *Leishmania* parasites, although usually this is associated with increased bite rate (Rogers and Bates, 2007). The evidence suggests that, albeit in mice, *L. major*-infected females of both vector sand fly species readily take a bloodmeal, (Ashwin et al., 2021) and *P. duboscqi* infected with *L. major* can cause cutaneous leishmaniasis in rodents (Teixeira et al., 2014) and non-human primates (Oliveira et al., 2015). However, it is unclear if this phenomenon will be reproduced in a CHIM study using *Leishmania*-infected sand flies.

Some participants were noted to have persistent but improving sand fly bite reaction at the final follow-up visit, and some were noted to have recrudescence of erythema towards the final days of data recording. Although all participants had stated that this had improved by the focus group visit, this was not formally recorded or measured. An extended follow-up period would have allowed for greater characterisation of this variation in response. A perceived future sequelae of this persistent lesion may be difficulty distinguishing between a sand fly bite reaction, and a developing *Leishmania* lesion given the usual time frame of natural lesion development, which may be acerated given the concentration of sand flies in the biting chamber (Khamesipour et al., 2005a), (El Hajj et al., 2004). Although the focus

groups confirmed positive outcomes, not all participants were able to attend to share their views, and it is possible that some additional insights were missed as a result. Furthermore, in contrast to the methodology used here individual interviews have been shown by some sources to provide greater understanding of participant knowledge (Morgan, 1996), although this is not universally acknowledged (McLafferty, 2004).

In conclusion, the successful completion of this underpinning study will contribute to further development of a CHIM model for leishmaniasis. The protocol, although now validated, based on these results will undergo some modification to allow suitability for a formal Leishmania CHIM (see chapter 4), where the translation of these methods will be tested.

4 Chapter 4 – Leishmania controlled human infection model

4.1 Introduction

4.1.1 Background

There is large spectrum of *Leishmania* disease depending mainly on infecting species, but also a range of other factors, including host response. Of the more than 20 species of *Leishmania* that cause human disease, *L. major* is widely thought to be associated with least long-term clinical sequalae. *L major* tends to cause single lesion disease which can self-heal without persistent parasite presence (Sghaier et al., 2022). There is effective treatment available (Aronson et al., 2016) and in contrast to other species of *Leishmania*, limited evidence of reactivation on immune suppression (Postorino et al., 2011; de Souza et al., 2017). Given that arguably the most important factor in the paradigm for a suitable CHIM development includes the knowledge of a treatable strain with relevance to human disease (Parkash et al., 2021c), *L. major* is a strong candidate. Given the genetic homogeneity between species and the knowledge of heterologous protection against species causing VL, this increases the use case for *L. major* within CHIMs and subsequent relevance to vaccine testing (Romano et al., 2015).

The history of CHIM models, and experimental infection using *Leishmania* on humans has been discussed at length in Chapter 1 and 3. A previous experimental *Leishmania* infection study conducted in Iran in 2005, made use of an *L. major* isolate produced according to the principles of good manufacturing practice (GMP) and used for both previous studies involving leishmanization, as well as a formal leishmanization-vaccination program during the Iran-Iraq war (Khamesipour et al., 2005a). Although well characterized, this isolate was obtained from an animal reservoir (*Rhombomis opimus*), however the World Health Organisation sanctioned its use in clinical trials, given the pressing public health need. There were some issues with this challenge agent, namely poor viability, repeated passage with associated loss of virulence, and persistent clinical lesions (Khamesipour et al., 2005a). As previously discussed, the use of sand flies, as the natural vector of leishmaniasis is important given the vector manipulates immunity, and needle vaccination is not necessarily

protective against further natural sand fly-initiated challenge. The natural evolution is therefore a sand fly-initiated CHIM for *Leishmania*.

4.1.2 Characterisation of a novel *Leishmania* isolate for use in a controlled human infection model

An important consideration for selecting an infectious agent for use as a challenge agent is to ensure that its provenance can be fully elucidated. This safeguards for example, against the presence of other blood borne infections from the parasite donor (including viruses, bacteria, parasites and prions) and in-vitro exposure to agents (e.g. bovine serum) able to cause transmissible spongiform encephalopathies (TSEs). Although many parasite banks can provide *L. major* isolates (ATCC 2023) no such lines of *L. major* that adhere to the above concepts are available. Although the leishmaniaviruses (discussed in chapter 1) are poorly understood and may have differing impacts for different species of *Leishmania* (Saura et al., 2022), there is a suggestion that they may in fact be arboviruses that can be acquired by animals, hence the use of parasite banks from non-human origin could be seen as questionable (Zhai et al., 2010). Additionally, given the risk of hybridisation, divergence and intra-strain differences which may be compounded when using strains taken from non-clinical samples, it is important that a challenge strain is well characterized and obtained from clinical specimens (Ravel et al., 2006; Sarkari et al., 2016; Kato et al., 2021).

As such, I was involved in developing a novel parasite bank at the University of York (Ashwin et al., 2021). Parasites were freshly sourced from new clinical cases of CL, from named patients who were followed-up to ensure cure and expected disease course. Parasites were obtained at time of diagnosis under aseptic conditions at the Department of Medicine, The Chaim Sheba Medical Center, Israel. *L. major*_MRC-02 was obtained from a patient presenting with two ulcerated papules (~ 1.5 cm diameter) on their leg and a small nodule on their neck approximately five months after exposure to sand flies in the endemic area of the Negev, Israel (see Figure 4.1). The patient elected for no treatment and there was complete healing 4 months later. At 18-month follow up, lesions remained healed with some scarring. The patient was seronegative for HIV, HBV, HCV and HTLV1.

*L. major*_MRC-02 was established at passage 1 at the Hebrew University using GMP compatible culture media. Foetal calf serum was obtained from a TSE-free certified source (Thermofisher). Cryopreserved stocks were maintained in liquid nitrogen and samples shipped to York and Prague for further characterisation and to the Contract Development and Manufacturing Organisation (Vibalogics, Cuxhaven, Germany). Phylogenetic analysis demonstrated the relationship between *L. major*_MRC-02 and other known strains, including the *L. major* Friedlin reference strain (see Figure 4.2).

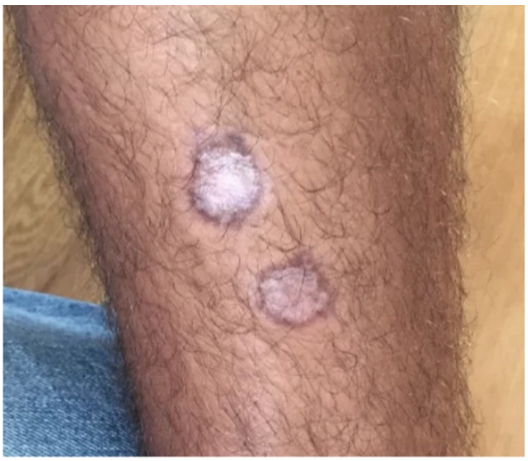

Figure 4.1: Figure showing clinical disease sustained by MRC_02 donor patient

Donor lesions for MRC_02 shown, patient photographs 9 months after travel to the Negev region, Israel. Photograph show lesions on the shin, which had been allowed to self-heal as per patient choice. Modified from (Ashwin et al., 2021).

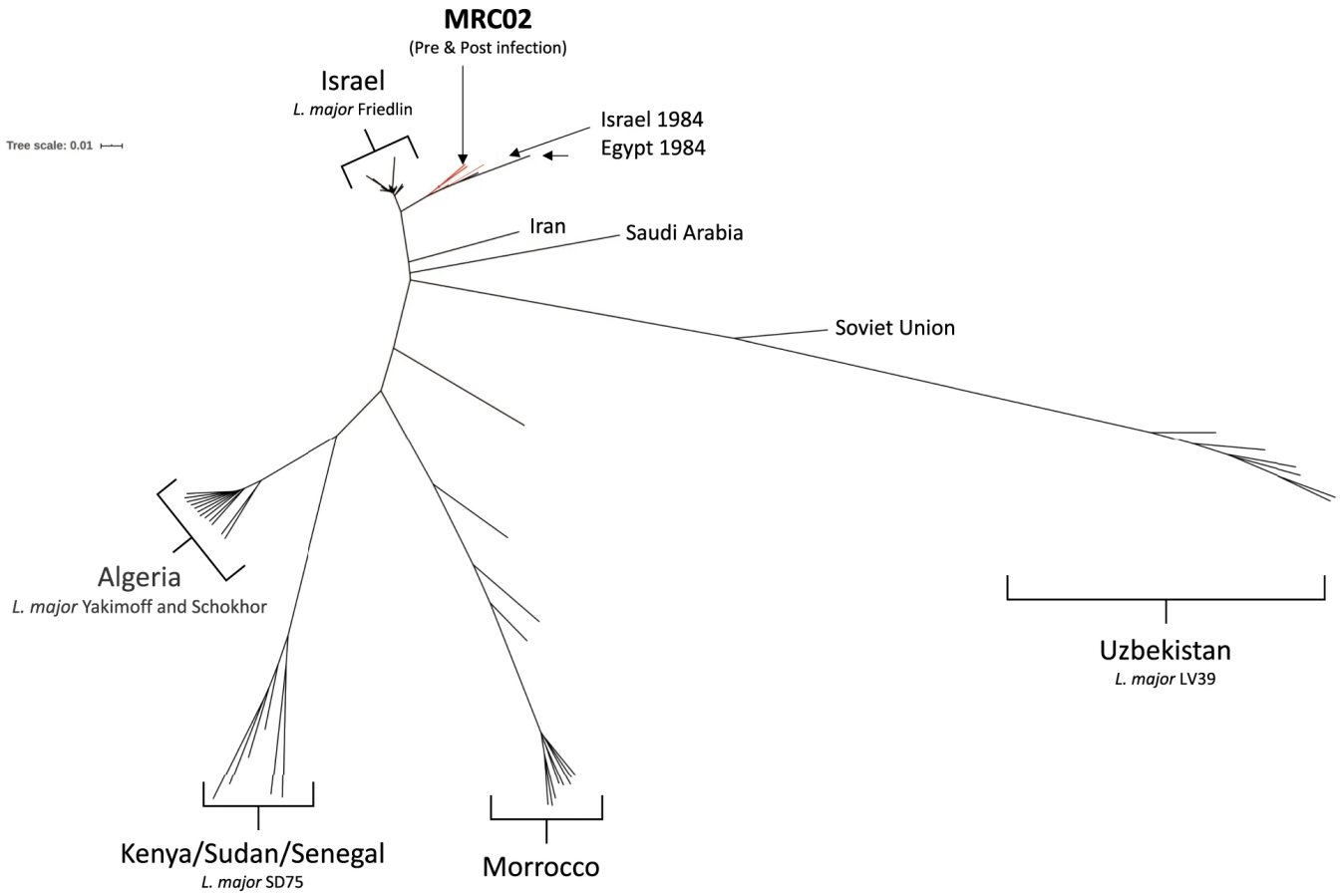

Figure 4.2: Phylogenetic tree for *Leishmania major* strains

Challenge strain MRC_02 placed within phylogenetic tree for known strains of *Leishmania major* originating from different geographical regions. *L.major* Friedlin, which is a known reference strain, is displayed, demonstrating close phylogenetic proximity to the challenge strain. Pre- and post-murine infection MRC_02 strain demonstrated. Figure adapted from (Ashwin et al., 2021).

L. major_MRC-02 was genotyped by next generation sequencing under contract at Genome Quebec, using DNA prepared at the University of York. DNA was also obtained from this line after passage in BALB/c. The data (i) confirm its identity as an *L. major* strain of likely Israeli origin, (ii) identified single nucleotide polymorphisms compared to the reference strain *L. major* Friedlin and (iii) confirmed that MRC-02 was genetically stable after a single passage in mice, based on both sequence and copy number variation. The GMP clinical parasite bank that has been generated is sufficient to conduct CHIM studies in >1200 individuals, including by sand fly transmission. Although the number of potential experiments with this strain is limited in comparison to comparable virally-vectored vaccines, the need to balance yield with limiting in-vitro parasite expansion predominates. This isolate manufactured to GMP, therefore gives option for needle challenge, given any needle-initiated infection will likely be required to conform to conditions pertaining to an investigational medicinal product (IMP).

4.1.3 Hypothesis

A sand fly-initiated *Leishmania major* controlled human infection model, will successfully demonstrate transmission to healthy volunteers determined by development of clinically compatible CL lesions and microscopic and molecular evidence of infection after skin biopsy. Skin biopsy is an effective method of treating and terminating the infection.

4.1.4 Objectives

4.1.4.1 Primary Objective

The primary objective is the development of a controlled human infection model of *Leishmania major* using sand fly transmission which is (a) effective and (b) safe.

4.1.4.1.1 Outcomes

Effectiveness were assessed by -

The take rate of parasitologically confirmed cutaneous leishmaniasis lesions in study subjects

Safety were measured by -

Assessing adverse event data collected through history, clinical examination & blood tests.

The development of any study-associated SAEs or grade 3 AEs at day 4 post-biting will result in a temporary halt and review of the sand fly biting schedule (see 4.2). Therefore, the Clinical Management Group (CMG) will review the safety outcomes 4 days after all biting procedures in real time for each pair of subjects (see 4.2).

Successful treatment of CL lesions in participants, and absence of lesions at 1 year follow up.

4.1.4.2 Secondary Objectives

1. Determine rate of CL lesion development following infected sand fly bite
2. Determine response to *Leishmania major*-infected sand fly bite in terms of immunohistology and immunopathology
3. Determine parasite load in CL lesions in comparison to number of sand fly bites received and rate of lesion development
4. Determine acceptance and psychological impact of *Leishmania major*-infected sand fly challenge

4.1.4.2.1 Outcomes

1. As determined by clinical examination, then biopsy and parasitological confirmation
2. Analysis of immune and inflammatory response (for example macrophage and T cell phenotype) and histology compatible with CL.
3. By PCR analysis of biopsy tissue of lesion site
4. Using psychometric questionnaires and focus groups

4.1.4.3 Exploratory objectives

In the case of a ≤66% take rate of *Leishmania major* with *Phlebotomus duboscqi*, to evaluate the take rate using *Phlebotomus papatasi*.

To deep phenotype and compare CL and normal skin biopsies, in those who agree to donate such, using digital spatial profiling of host and parasite mRNA and protein expression, and mass spectroscopy imaging.

To determine human reactogenicity to *Leishmania major*-infected sand fly bite in macroscopic, dermoscopic, immunological and biochemical terms

4.2 Methods

4.2.1 Study Design

This is a clinical study in up to 18 healthy *Leishmania*-naïve subjects aged between 18 and 50 years old who develop a confirmed sand fly bite. The study had an adaptive design with the initial plan to enrol six subjects and expose them to biting by *Phlebotomus duboscqi* infected with *Leishmania major*. The adaptive design was pragmatically designed to minimise unnecessary exposure of volunteers to *Leishmania* and maximise the likelihood of developing a reproducible CHIM (See Figure 4.3).

The clinical study and clinical research was carried out at the Translational Research Facility (Q Block), HYMS / Department of Biology, University of York, York, YO10 5DD. Routine laboratory investigations were carried out at York Teaching Hospitals NHS Foundation Trust, Wigginton Road, York. The focus group(s) will take place at the University of York, York, YO10 5DD (or virtually if in-person meetings are not possible).

Professor Charles Lacey was the Chief Investigator for this study entitled LEISH_Challenge, and I was a Principal Investigator, leading on the clinical aspects and overall running of the study.

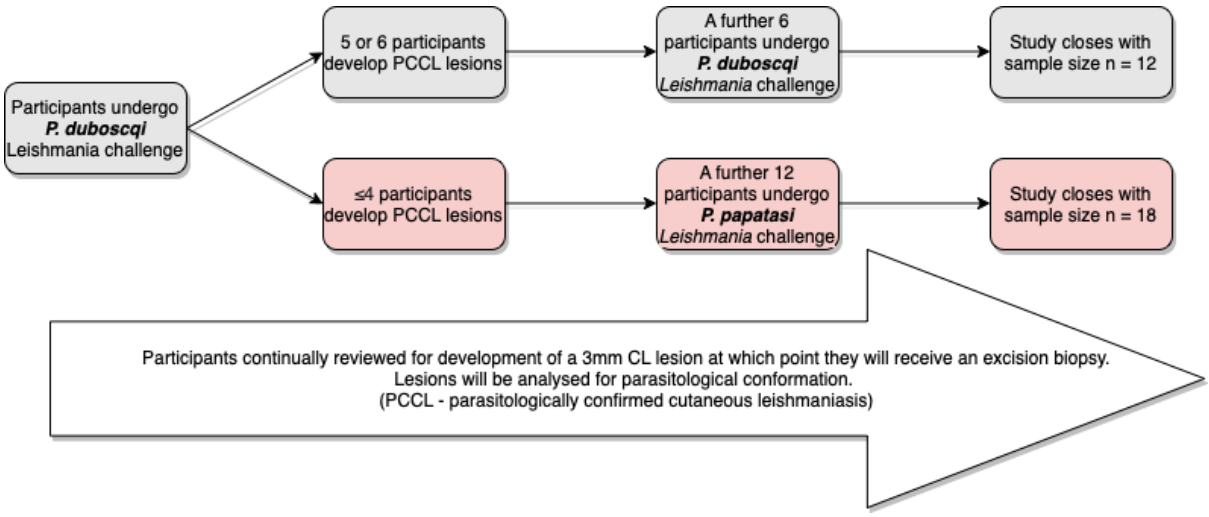

Figure 4.3: Study schematic

There was ongoing clinical review of participants to monitor the development of an early CL lesion. If either 6 or 5 participants had developed PCCL lesions within the 6-month follow-up after *Leishmania* challenge, then a further 6 subjects would undergo *Leishmania* challenge by *P. dubosqi*. If only 4 or fewer subjects in the first cohort develop PCCL lesions, then switch to an alternative vector would be considered, (i.e. *P. papatasi),* and a further 12 subjects would undergo *Leishmania* challenge.

4.2.2 Sample size considerations

The development of controlled human infection model for *Leishmania* has parallels with other historical CHIMs, for example, the first description of a malaria CHIM in the modern era using mosquito transmission, which was in four subjects (Herrington et al., 1988) and the first modern blood stage *Plasmodium falciparum* CHIM employed five subjects (Cheng et al., 1997). Sample size calculations for this CHIM consider several factors, including sample sizes in pilot studies in the development of other CHIMs, with subsequent successful candidate vaccine evaluation, such as those concerning *P. falciparum* (Whitehead et al., 2016). (See also chapter 3).

One of the considerations is the need to determine an effective take rate which would feed into calculations for any *Leishmania* vaccine-CHIM, whilst counterbalancing the need for safety to be prioritised. As a result, an adaptive design was chosen with an initial smaller enrolled cohort, and then depending on success, further participants were to be enrolled. Determining potential sample size with a dichotomous end point (i.e. lesion development vs no lesions development) compared to a continuous variable is challenging, without a placebo arm. This is common with many other novel CHIMs in the initial phases where specific sample size calculations are not undertaken, but sample sizes are in single to double digits and are decided based on the probability of meeting the primary objectives (Killingley et al., 2022).

Therefore, in conjunction with study statisticians it was determined that in any formal *Leishmania* vaccine-CHIM which was 90% powered to detect a vaccine efficacy of 60% and a reduction in lesion development, then for a take rate of 100% in the placebo arm (6/6 participants), 22 participants would be required in the vaccination arm. This number rose to 62 participants for a take rate of 67% in the placebo arm (4/6 participants). For a vaccine efficacy of 50%, the sample size would be 28 and 90 respectively. These figures assume a $p<0.05$. Therefore, the sample size in this study was deemed to be a total of 12 participants to allow evaluation of the take rate, which was higher than other pilot CHIM studies, with the possibility of replacing participants who had experienced 'biting failure' if needed (see 4.2.14).

4.2.3 Sand fly development

See section 3.2.5 for sand fly transport, development and related procedures.

4.2.3.1 Sand fly feeding for *Leishmania* CHIM

Once the sand flies arrived at the Department of Biology, University of York, a cotton-soaked sugar solution (50%) was placed within the cage for 24 hours, from which the sand flies could feed. The sugar solution was then removed after 24 hours and the sand flies were starved. Twelve to fifteen days prior to a scheduled biting day, sand flies were infected with *L. major* using a membrane feeder (Hemotek) containing rabbit blood mixed with 10^6 / ml promastigotes of *L. major*_MRC-02. Female sand flies prefer to feed in the dark, hence the insect cage was covered with an opaque material during this process. Three to five days before a scheduled biting day, a subset of the engorged sand flies were killed and dissected to evaluate infection rates by standard methods. If infection rate was below 90% or parasites had failed to develop to infectious forms, the study was postponed.

On the day of the biting study, the biting chambers were loaded with infectious sand flies within the secure insectary. Sand fly containers were cooled with surrounding ice (as cold temperature reduces the metabolism of the sand flies and immobilizes them) allowing ease of handling. Five female previously engorged female sand flies where then added to the biting chamber using tweezers. If sand flies were not available on the day of the biting study for any reason, the study was postponed until the sand flies were available.

A record was kept of each lot of sand flies used during the study. This includes the participants' study number, the description (lot numbers) of sand flies received at study site and date of receipt, as well as a record of when (date of biting challenge) and whom (volunteer number) underwent challenge.

4.2.4 Study Volunteers and biting procedure

Healthy participants aged between 18-50 years were recruited (see Figure 7.20: LEISH_Challenge advertisement posters). All subjects were willing and able to adhere to the study conditions, methodology and to give written informed consent. Participants underwent the biting procedure with 5 sand flies over 30 minutes. Only one study participant underwent sand fly biting at any one time. A custom biting chamber was used (see section 3.2.6).

The sand fly biting chambers were secured on the participants arm for 30 minutes after which it was removed. The participants were observed both during the 30-minute biting period and for 2 hours after for signs of any localised and/or generalized reaction. A medical team was present at all times. This consisted of at least 1 doctor and 1 nurse. In the unlikely event of a nurse not being available (e.g. due to NHS surge pressures) an additional medically qualified staff member (i.e. doctor) was available instead. See section 3.2.9 for further discussion on resuscitation equipment.

Localised skin reactions including redness, scaling and swelling could be treated with oral antihistamines (such as cetirizine, chlorphenamine) for pruritus (itch) or any persistent reaction. Any pain or discomfort would be treated with oral paracetamol and/or ibuprofen. The biting site was covered with a light dressing if necessary. These medications were available from the Translational Research Facility after being prescribed and dispensed by a medical practitioner on the study team.

4.2.5 Safety review within study

Only 1-2 participants could undergo sand fly biting on any given day, and no participants could undergo sand fly biting simultaneously. Participants were scheduled to attend for further follow-up post-biting visit (Table 4.1, 4.2 & 4.3). The development of any SAE or non-solicited grade 3 or greater AE at the follow-up visits, which are considered possibly, probably or definitely related to the biting procedure would result in a temporary halt and review of the sand fly biting parameters. Therefore, the safety outcomes were reviewed 4 days after all biting procedures in real time for all subjects. If an SAE or non-solicited grade 3

AE had been recorded the CI (CL) and PIs (VP, AL & PK) would review both the clinical event, and the biting parameters in terms of the number of sand flies, the species and length of exposure, with regard to progression of the study. If non-solicited Grade 3 / exaggerated reactions had been observed it would be likely that the Investigators (as above) would seek a protocol amendment to decrease the number of sand flies present within the biting chamber, or length of exposure to mitigate any exaggerated response. At the discretion of the clinical investigators/CMG (CL, VP, AL) it would be possible that a sufficient number of grade 2 AEs, would also result in such a halt to the study and consideration of a protocol amendment.

Any diagnosis of an intercurrent illness during the study, which is not related to the study or its procedures, will either be referred to the subject's GP, or if necessary, the Emergency Department at York Hospital.

4.2.6 Inclusion and Exclusion Criteria

This study was conducted in participants who met the following inclusion and exclusion criteria.

Inclusion Criteria:

The participant must be:

- Healthy adults aged 18 to 50 years on the day of screening
- Willing to give consent for exposure to Leishmania-infected sand fly with the intention of causing a cutaneous leishmaniasis lesion
- Willing and able to give written informed consent
- Willing to undergo Hepatitis B, Hepatitis C & HIV testing
- Willing to undergo a pregnancy test during screening and follow-up visits and must not be breastfeeding (pre-menopausal female participants)
- Willing to refrain from blood donation during the study
- Using a reliable and effective form of contraception (pre-menopausal female participants up to 3 months post-biopsy

- Judged, in the opinion of a medically qualified Clinical Investigator, to be able and likely to comply with all study requirements as set out in the protocol
- Without any other significant health problems as determined by medical history, physical examination, results of screening tests and the clinical judgment of a medically qualified Clinical Investigator
- Available for the duration of the study
- Willing to refrain from travel to regions where Leishmania-transmitting sand flies are present, from recruitment until an appropriate point (judged by study investigators).
- Willing to consent to a copy of the past medical history to be provided by the participant's GP practice.
- Agree to registration on a national database of study & trial subjects to prevent over-volunteering (TOPS)
- Willing to give consent for study investigators to contact the participants GP in the event of a significant abnormality being observed
- Willing to show identification documents to confirm identity
- Willing to give consent to biopsy(s) of suspected cutaneous leishmaniasis lesions

Exclusion Criteria:

The participant may not enter the study if any of the following apply:

- Receipt of any vaccine within 21 days of screening. Participants must also agree to avoid all immunisations in the 21 days, both before and following the Leishmania challenge procedure.
- Administration of immunoglobulins and/or any blood products within the three months preceding the planned study.
- History of significant allergic disease/atopy (e.g. significant/severe eczema, hay fever, asthma) or reactions; or a history of severe or multiple allergies to drugs or pharmaceutical agents, as judged by the clinical investigators
- Any significant chronic skin condition as judged by the clinical investigators
- Any history of confirmed Leishmaniasis infection
- Any history of travel (within the 30 days prior to the Leishmania-infected biting visit) to regions where Leishmania major-transmitting sand flies are endemic*.

- Any history of more than 30 continuous days stay in regions where Leishmania major-transmitting sand flies are endemic within the last 10 years*.
- Any history of severe local or general reaction to insect bites, defined as
- Local: extensive, indurated redness and swelling involving most of the antero-lateral thigh or the major circumference of the arm, not resolving within 72 hours
- General: fever ≥ 39.5°C, anaphylaxis, bronchospasm, laryngeal oedema, collapse, convulsions or encephalopathy within 48 hours
- Any history of anaphylaxis
- Females – current pregnancy, less than 12 weeks postpartum, lactating or willingness/intention to become pregnant during the study.
- Any clinically significant abnormal finding on screening biochemistry or haematology blood tests as judged by study investigators
- Total IgE levels > 214 IU/ml
- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months
- A diagnosis of diabetes type 1 or type 2 or significantly raised HbA1c
- Active Tuberculosis, leprosy, or malnutrition
- Any significant chronic illness requiring hospital specialist input as judged by study investigators
- Any significant psychiatric conditions as judged by general practitioner and/or study clinical team
- Unlikely to comply with the study protocol
- Participating in significant current or recent research (involving an investigational medicinal product or other significant intervention) within the past 3 months (as judged by study investigators)
- Any other significant disease, disorder, finding or medical history, which, in the opinion of a medically qualified Clinical Investigator, may either put the participant at risk because of participation in the study, or may influence the result of the study, or the participant's ability to participate in the study
- Any known risk factors for Creutzfeldt Jakob Disease (CJD) or variant CJD

*This refers to regions where Leishmania major-transmitting sand flies are endemic including (but not limited to) the Middle East, Sub-Saharan Africa, and Asia. (see Figure 4, World Health Organisation Map)

4.2.7 Screening Procedures and Investigations

The following general eligibility criteria were assessed prior to conducting informed consent during a pre-screen:

- Age
- Availability for the duration of the study (including follow-up)
- General health state (including history of atopy i.e. asthma / hay fever / eczema)
- Allergy status (medications and other allergens)
- Risk of previous Leishmania and phlebotomine sand fly exposure as determined by travel history
- Contact details

This pre-screen assessment could take place face-to-face, via phone or via email (see appendix Figure 7.19: LEISH_Challenge pre-screening questionnaire). Following the pre-screening assessment, the participants were given written material (see Figure 7.21) and allowed to consider their recruitment into the study. A discussion of the potential risks and obligations were also explicitly highlighted. If the participant indicated a willingness to participate, they were assigned a date and time for a formal Screening Visit. It is possible that a screening visit could take place concurrently if the participant had enough time to read the recruitment material and make an informed decision based on these materials.

If the participant was still willing and interested, they were be asked to sign and date a copy of the Consent Form (see appendix Figure 7.22). A copy was given to the participant to keep, a copy was stored in the participant's case file and the original was placed in the Study Site File.

To ensure informed consent, the following information was provided by a member of the study team at screening

- Pre-HIV test discussion
- The importance of continued follow up in the study to monitor any unforeseen events were stressed

After informed consent had been obtained, assessments and investigations were undertaken according to the schedule. These included:

- Medical history
- General physical & skin examination
- Confirmation of identification and photography
- If female, a pregnancy test
- Blood samples for routine laboratory investigations (haematology and biochemistry) and the blood borne viruses Hepatitis B, Hepatitis C and HIV.
- Blood sample for *Leishmania* antibody test and immune monitoring
- A standard letter was sent to the GP outlining the study (see appendix Figure 7.23). The GP practice were then asked to provide a summary of the medical history.

A record of the above procedures and any findings were entered into the Case Report Form (CRF)

Each participant who entered the study by signing a copy of the consent form was assigned an Identification Number. These numbers were not reassigned. A screening log of screened participants with their identification number and basic details was maintained and kept in the study site file to track participants who have been screened for the study.

Following the screening visit I reviewed the results of each participant to determine if they were eligible for further involvement in the study. This was confirmed by another medically qualified member of the team for each participant.

Participants were screened up to 90 days before the biting day visit. If this 90-day period elapsed, participants could still be included in the study if there have been no significant changes to medical history or circumstances, and a new set of baseline blood tests had been obtained within 90-days of the biting visit. If there has been a significant change in

circumstances, then participants could take part in the study only after a full re-screen had taken place.

4.2.8 Eligibility and Screening failures

I confirmed eligibility for each participant based on the screening procedures, including findings from clinical histories, examinations laboratory results. If the participant was deemed to be eligible, then further participation could be undertaken.

If for any reason the participant was considered a screen failure I informed them of this including a summary their results and the reason for the screen failure.

4.2.9 Repeat screening

If one or more of the laboratory screening test results were deemed to be an anomaly and temporary (as described in MHRA Good Clinical Practice guidelines; 1st Edition 2012, Section 11.4.3 - page 377), then a repeat of the relevant tests were carried out. The reason for carrying out a repeat testing was clearly documented and subjects may could only undergo repeat testing to confirm or dispute anomalies. If, following repeat testing, any laboratory parameter continued to fall outside of the inclusion range for the study then the subject was be excluded from the study and no further re-screening could take place. If an anomaly was reported as a result of lab error, then this did not count as a repeat test.

4.2.10 Pregnancy

If a participant is found to have a positive pregnancy test at the screening visit, they were considered a screen failure. The participant would be given the result by myself, and a report sent to their GP. They could still be able to take part if they expressly wish for this at a later date, but only if they attend for repeat screening and a full assessment made on eligibility (e.g. false positive pregnancy test or after termination of pregnancy).

Pregnancy testing was performed by use of serum β-human chorionic gonadotropin (β-HCG) result at the screening visit. Urinary pregnancy testing (urinary β-human chorionic

gonadotropin) was used if a suspicion of pregnancy arose during the study. If the following serum β-HCG values were resulted, this would prompt further investigation:

- Females pre-menopause: ≥ 1 IU/L (could suggest pregnancy)
- Females post-menopause: ≥ 7 IU/L (could suggest other pathology that would need follow-up)

Reference ranges were advised by the testing laboratory (York Teaching Hospitals NHS Foundation Trust).

If a female participant becomes pregnant following infected sand fly bite, she were followed up as other participants. The pregnancy would be reported to the Sponsor in accordance and a report sent to their GP. There are documented potential negative sequelae of cutaneous leishmaniasis in pregnancy (Morgan et al., 2007).

4.2.11 Baseline and continuous psychological assessment and wellbeing

Psychological well-being was evaluated at baseline and at regular intervals during selected visits (Table 4.1, 4.2 & 4.3). This was undertaken to determine acceptability of the study and its procedures to the participant, and whether participation was associated with any undue psychological outcomes. Two instruments were used, the GAD-7 psychometric questionnaire and quality of life measure (Toussaint et al., 2020) and the DLQI (Dermatology Life Quality Instrument) (Basra et al., 2015). These questionnaires could be filled remotely using the online diary card, or on paper if the participant preferred (see Figure 7.25).

The GAD-7 is a brief 7 item generalised anxiety measure with normative data available. It has good thresholds and cut-off values and were able to capture the key generalised anxiety components that were identified as possible issues in the FLYBITE study. The DLQI is a validated generic dermatology quality of life questionnaire and gives more capacity to collect other patient reported psychosocial dermatological symptoms. Both scoring systems used a brief questionnaire which allowed for ease of use with the aim to ensure compliance and reduce fatigue of participants with respect to study procedures.

4.2.12 Study procedures

Procedures were performed on the visit time points indicated in the schedule of procedures. Additional procedures or laboratory tests could be performed, at the discretion of a Clinical Investigator if clinically required.

Observations included blood pressure, pulse rate, temperature, oxygen saturations and respiratory rate. Height and weight were also be recorded at screening and a repeat taken during the study only if necessary.

Blood tests were taken for the following laboratory tests at visits as per the schedules:

- Haematology: Haemoglobin, White blood cells, Neutrophils, Lymphocytes, Platelets

- Biochemistry: Renal function test (urea and electrolytes), liver function tests, C –reactive protein, HbA1C

- Blood Borne Virus Screen: Hepatitis B surface antigen, HIV antibodies, Hepatitis C antibodies

- Immunology: Total IgE, CD4 & CD8 subsets, immune monitoring

- Cellular responses: peripheral blood mononuclear cells isolation

- β-human chorionic gonadotrophic: in women at screening

- *Leishmania* & sand fly testing: Serology and other confirmatory tests to determine presence of *Leishmania* and sand fly-related factors

Urine was only collected for dipstick testing for diagnosis and management of intercurrent medical presentations during follow up, e.g. systemic illness, UTI, suspicion of pregnancy, etc:

General examination was performed and included cardiovascular, respiratory, neurological, abdominal, inspection of proposed bite site and palpation of axillary and cervical lymph nodes. A general examination of the skin to exclude relevant inflammatory dermatoses was also undertaken.

Haematology, biochemistry, blood-borne virus testing and IgE were tested at the York Teaching Hospitals NHS Trust. Blood taken for these tests were be transferred usually within 24 hours to the hospital laboratory. In exceptional circumstances, samples could be temporarily stored at the University of York prior to transfer to the York Teaching Hospitals NHS Trust for testing.

4.2.13 Study visits

See Table 4.1, 4.2 and 4.3 for schedule of visits from screening to biting and from biopsy to end of study.

4.2.13.1 Visit 1: Pre-screening, screening and Enrolment

All potential participants had a pre-screening assessment conducted either face-to-face, via email or via telephone to determine eligibility and availability. I assessed their eligibility if they fulfilled basic criteria for progression to screening, the study was discussed with them, and information leaflets provided (See appendix Figure 7.19, 7.20, 7.21).

4.2.13.2 Visit 2: Sand fly biting visit

A further brief assessment of eligibility was conducted at the beginning of this visit, prior to the sand fly biting which included a further consent process to check understanding of the study and risks. The importance of continuing to attend follow up visits to monitor any unforeseen events was again stressed to the participant

The participants were asked to avoid wearing deodorants, aftershaves and perfumes on the day. Participants were be asked to wash with a neutral scent-free wash on the day, prior to attending for sand fly biting visit (This was be provided at the screening visit). They were under no obligation to use this wash.

The placement for each biting chamber (containing the sand flies) was on the volar aspect of the forearm, approximately 2-3 centimetres distal to the antecubital fossa. This site was chosen for both cosmetic reasons and reduced likelihood of scarring and will therefore be recommended to participants. Either arm can be used, but the use of the non-dominant arm was suggested. During the FLYBITE study, all participants agreed to the volar aspect of the forearm as an agreed area for sand fly biting, hence it was anticipated that this site would be acceptable to all the participants.

The selected site was examined to ensure suitable skin quality and photographed prior to placing the biting chamber. Photography was repeated over the course of the study. Video recording were employed to record sand fly feeding behaviour but limited to the biting chamber, and not so that the participant could be identified. The area around the biting chamber were marked with a suitable marker pen. This will ensure that visual and dermatoscopy inspection can occur accurately at the bite site and during follow-up. Participants were asked to mark this area with a pen that were provided, if they are happy to do so in order that the biting region can be readily identified at follow-up and in pictures. Data relating to sand fly behaviour was recorded. Participants' sand fly biting experiences were also recorded. This include any features such as pain and itch, recorded using a visual analogue score is feasible that participants may give a video account of their experiences, although this were optional and the participant will have the choice to for this to remain anonymous (i.e. any recognisable features were hidden.

All participants were issued with a study participant identity card and encouraged to contact the research team if there are any problems.

Participants were required to stay within the TRF for 2 hours after the end of sand fly biting procedure. During this time, observations (including temperature, heart rate, blood pressure, respiratory rate) were be performed at 30 minutes intervals. Further observations were taken if there were any significant issues either reported by the participants or observed by the clinical team. During this period further information such as number of visible bites and dermoscopy were also recorded. A further clinical review was taken place prior to the participant leaving the unit. Over-the-counter antihistamines (cetirizine) and simple analgesics (paracetamol and ibuprofen) were available if the participant wished to take these the event of a significant localised reaction at the bite site e.g. itch.

Up to two participants were studied each day of biting, with one in the morning, and one in the afternoon. The biting days were dependent on availability of sand flies and other logistical features. The biting days took place on weekdays only and within 'business' hours.

4.2.13.3 Diary Cards

Participants were given a diary to record both local (cutaneous) and systemic symptoms from the biting visit until the final follow-up visit using a Visual Analogue Score (VAS). All participants used the option for a secure online electronic diary card, although the option for a paper diary card was available to all participants. The diary card included the option to upload pictures of the lesion at designated time points, or if the participants wished to. The use of online methods of data capture was discussed with the University information controller and other relevant departments.

This did not preclude participants reporting any adverse reactions directly to study investigators or by the other means outlined in this section. There was space to include additional relevant information including new medications and use of any over-the-counter medications (including those prescribed by study clinicians).

An alert card was provided which contained information about the study for the attention of health care professionals, in case urgent medical care is needed. It also contained

urgent/emergency contact numbers for both the study clinicians and for NHS services (see appendix Figure 7.24).

A ruler (Cohort 1) and a digital calliper (Cohort 2) was provided to record the size of the lesion when photography was uploaded by the participant.

Diary card data in of its own did not constitute an adverse event, but aided investigators in determining presence of AEs. Presence of any significant or non-solicited grade 3 or higher AEs or any SAEs recorded on the diary card, as judged by investigators, were reported to the Sponsor.

4.2.13.4 Follow-up Visits

Further assessments took take place at 4, 14, 28, 42, 56, 70, 98, 126, and 154 days after the biting visit. Visits 3, 4, 5, 7, 9, 11 (numbering as per schedule) took place in person in the TRF, whereas Visits 6, 8, 10, are scheduled to be 'remote visits' conducted by Video Calling. These can be changed to in person visits if there are any clinical problems, or if the participant wished. The windows of compliance with the protocol for these visits are indicated in the schedule.

Participants were assessed for local and systemic adverse events using a focused history, physical examination, dermoscopy and photography. The presence or absence of lesion was recorded, and the size of a lesion was measured. It was anticipated that an early lesion may be observed clinically but may not be measurable accurately in terms of size.

Participants were asked to take digital pictures of the lesion on an up to twice weekly basis (or greater if necessary and agreed to by the participant), although this were optional. The frequency of photography uploads was decreased depending on participant progress and following biopsy. Participants were asked if they were happy to take these pictures with their own mobile phone camera (depending on the quality of baseline imaging as determined by study investigators). Alternatively, a digital camera could be provided to facilitate this. Participants were encouraged to electronically upload these by using the

secure diary card after they had been taken, or else provide them at the next study visit. Further imaging was taken by study investigators at study visits, including using dermatoscopy.

Participant photography allowed study investigators to monitor the development and/or progression of any lesion. It aided in the decision by the study investigators to attend for a further study visit if needed. Given that the development of a CL lesion may take some time, it was anticipated that this process will negate the need for frequent face-to-face follow-ups whilst also capturing useful data that may not be gleaned without daily follow-up. This was especially relevant given the risks to individuals of COVID-19, which was deemed to be a risk during the development of this study.

Follow-up visits focused on the reactogenicity experienced by participants and the potential development of a CL lesion. If a lesion developed which was ≥ 3mm in diameter and had clinical characteristics of a CL lesion the subject exited the 'passive follow up' phase and enter the biopsy & treatment phase of the study (see section 4.2.13.6).

4.2.13.5 Visit 3 to Visit 11

See schedule of visits (
Table 4.1 & Table 4.2) for anticipated visit related activities.

4.2.13.6 Potential development and clinical diagnosis of a cutaneous leishmaniasis lesion

It was anticipated that from Visit 4 onwards a subject could develop an early CL lesion at the bite site.
The following criteria was used to determine the presence of a clinically compatible cutaneous leishmaniasis lesion, following successful sand fly biting:

If a lesion is present at 14 days (visit 4) or more post-sand fly biting, which is papular, raised, erythematous and ≥3mm in diameter, and clinically compatible with a CL lesion as judged by

study investigators, then the participant were deemed to have a clinically compatible cutaneous leishmaniasis lesion.

Procedures and follow up subsequent to clinical diagnosis of a cutaneous leishmaniasis lesion are summarised in Table 4.3: Schedule of Visits, Treatment Phase, from day 0, the day of biopsy.

4.2.13.7 Biopsy visit

If a lesion fulfilled these characteristics, it was excised using a formal excision or punch biopsy, according to an excision biopsy standard operating procedure (see Figure 7.32 and Figure 7.34) and analysed for parasitological confirmation, again according to a defined SOP (see Figure 7.33). The biopsy could take place from visit 4 onwards.

The biopsy procedure was carried out in the TRF. It could either be conducted at the same time as a routine follow up visit where the diagnosis was made, or at newly arranged visit. Risks as per all biopsies to include a small risk of infection, bleeding and pain as well as scarring. A specific biopsy consent form was completed (see Figure 7.26).

4.2.13.8 Follow up post-biopsy

Study subjects were followed up 10 days after biopsy / biopsies for review and suture removal. There were further planned follow-ups at days 30, 60, 90, 180, and 360 post-biopsy. If any lesions developed during follow up at, or near the original CL biopsy site, that are clinically compatible with recurrent cutaneous leishmaniasis, they were confirmed by biopsy and PCR and treated according to relevant guidance with treatment such as cryotherapy (Asilian et al., 2004; López-Carvajal et al., 2016). If further treatment was given for recurrent CL, then follow ups at day 90, 180, and 360 after the last treatment was performed, took place. The follow ups at days 90, 180, and 360 confirmed on-going cure, and are of similar duration to the recommended follow ups at 3 months and 1 year post-treatment of clinical CL (Blum et al., 2014). For any unexpected issues in clinical management, the participant would be referred to an appropriate NHS specialist. Some of

these visits could take place virtually, if there were no significant concerns expressed by the participant or clinical investigators.

4.2.13.9 Focus Group(s)

A focus group (or groups) with all the participants from the challenge study is planned approximately one year after the last subject successful biting visit. This were done using similar methodology which was utilised successfully in the non-infected sand fly bite study (see section 3.2.8.4). Because of the design of the study with (a) variable numbers and (b) the variable lengths of time taken to complete the study, as well as the length of the study, it is not possible to be precise as to the numbers of subjects that may be available for participation in the focus group. However, if 4 or more participants are available to take part in a focus group then this will take place. It may also be more pragmatic to hold two, or even potentially three focus groups. Such focus groups were at times that are mutually agreed with study participants and clinical staff. The decisions on the timing and numbers of subjects in the focus groups were made by the study team in conjunction with the health psychologist (Prof Georgina Jones). The ideal number of participants in each focus group is 4 – 8 participants(Liamputtong, 2015; Caroline Tynan and Drayton, 1988). The focus group(s) will take place at the University of York, York, YO10 5DD. It is feasible that the focus group(s) could take place virtually if an in-person meeting is not possible.

The aim of the focus group were to elicit the participant's experiences of participation in the CHIM in greater depth and gather information to help inform the design any subsequent leishmania CHIM studies. The focus group were digitally recorded (with consent) and it is anticipated that the focus group will last between 2-3 hours based upon the initial public involvement activity and the FLYBITE focus group. The focus group were fully transcribed verbatim and analysed using NVivo software (QSR International Pty Ltd). An inductive content analysis approach were employed (Elo et al., 2014). To establish the trustworthiness of the analysis, one member of the team will independently read the transcripts line by line and identify emergent themes (Noble and Smith, 2015). A second member of the team will independently check a proportion of these (50%) to verify the coding. Discussion of, and agreement upon, common patterns and broader themes from the participant's experiences

were reached between these colleagues. Any dissident views and areas of diversity were considered and discussed with the wider study team.

Participants were asked about their experiences of taking part in this study, centred on the following points:

(a) The recruitment process (to include screening and pre-screening)

(b) The procedures involved in the *Leishmania* challenge visit itself

(c) The experience of being bitten, and the subsequent evolution of the lesion

(d) The follow-up visits (to include testing procedures/photography)

(e) The biopsy procedures

(f) The topical treatment used

(g) Any safety concerns and if so, how the participants felt about the processes in place to mitigate these

(h) If taking part in this study is what the participants expected and if not, what was different or unanticipated

(i) Potential improvements for subsequent studies

(j) Any other discussion points raised by participants

It is feasible the focus group(s) may take place virtually using video conferencing technology. This will only occur after discussion with the wider study team, and if there are significant issues in availability, or other issues including, but not limited to COVID-19 outbreaks.

4.2.13.10 Additional Visits

Additional visits and assessments may be required to evaluate an adverse event and/or to identify a diagnosis. As per normal clinical standards of care, a further biopsy might be necessary to provide a differential diagnosis, for example later during the treatment phase of the study.

4.2.13.11 Virtual visits

Participants were able to conduct select visits via phone or email at the discretion of investigators. This was only possible if no complications were noted, and if the participant had been compliant with the study schedule up until that point. Evidence of compliance included attending for blood tests and sharing diary card and participant-recorded photography in a timely manner. This virtual visit was possible so long as the interval between face-to-face visits did not exceed 3 months (except the final visit which may take place virtually at the discretion of study investigators). It was feasible that video calling be used to conduct some virtual visits. An NHS accredited app such as Nye (https://www.meet.nye.health) could be used to conduct such a visit or other widely available technology such as Zoom. In lieu of these, it was possible to conduct visits by telephone if there were specific reasons that video technology could not be used.

4.2.13.12 Failure to participate in a visit

If a participant failed to take part in a study visit, in the first instance a repeat visit was offered within the visit window. If the participant was still unable to attend this visit, they will not be excluded, but a further visit was offered as close as possible to the visit window. If this occurs on more than one occasion for any given participant, it was then be deemed a protocol deviation, and a file note prepared and submitted to the Sponsor.

4.2.14 Suspected sand fly biting failure and participant replacement

The evidence for sand fly biting includes -

(a) Participant-reported biting sensation during, and immediately after biting

(b) Suspected sand fly biting activity noted by clinical investigators (including video and photography during biting)

(c) Presence of bite compatible lesions by dermoscopy or photography immediately after biting

(d) Presence of a dilated abdomen in any of the sand flies in the biting chamber following withdrawal of biting chamber from participant.

If none of b, c, or d were observed this would strongly suggest absence of a successful bite. Visits 3, 4, & 5 out to 28 days would still be completed, to check whether any later changes at the site indicated that successful biting had occurred. If no changes were observed the participant would be deemed to have failed biting, and further clinic visits would cease. We would organise a later Video Calling follow up at 6 months to check on the participant's welfare. Participants were able to contact the team if they had any issues in the interim.

If sand fly biting failure was determined in any participant at the 28 day follow up (visit 4), an additional volunteer could be recruited, so that the size of the cohort in follow-up need not be limited by biting failure. Such an additional volunteer was assigned a unique identification number. The subject & GP was provided with a specific information leaflet and were instructed to contact the study team should any lesion(s) subsequently appear (see appendix Figure 7.29 and Figure 7.31)

If a subject had experienced successful sand fly biting (as defined above) but failed to develop a lesion at the bite site during follow up out to visit 11 (day 154), then that would constitute the last formal study visit. The subject & GP was provided with a specific information leaflet (see appendix Figure 7.28 & Figure 7.30) and were instructed to contact the study team should any lesion(s) subsequently appear.

4.2.15 Failure of parasitological confirmation of a suspected cutaneous leishmaniasis lesion

If a subject developed a suspected CL lesion that was biopsied, but then the parasitological investigations were negative, then the investigators reviewed the clinical and histological data, to decide on a presumptive diagnosis and any necessary management. Formal follow-up in terms of the study would cease at that point if deemed not be cutaneous leishmaniasis, but additional visits in terms of alternative diagnoses would be carried out as indicated.

4.2.16 Safety of staff and participants during visits

This study was designed at the height of the SARS-CoV-2 pandemic and therefore a number safety features were incorporated into the conduct of this study. This was in order to ensure safety of both participants and study investigators:

- All participants were given allocated time slots to attend and overlaps between participants were avoided.
- Participants attended via a dedicated entrance to the building which led directly into the clinical area and were not used by non-study staff during study visits.
- The clinical area was not accessible by non-study staff during the study visits.
- All surfaces and equipment were cleaned using an appropriate disinfectant between participant visits, including the sand fly biting chamber (e.g. Tristel Fuse or similar product).
- When examining and during contact with participants, disposable gloves and an apron were used by study investigators.
- Surgical masks and/or visors were used in accordance with NHS and research practice depending on the latest government advice at the time of the study.
- Participants were encouraged to wear face coverings in accordance with any national guidance.
- The minimum number of study investigators present at any one time with the participant to ensure adequate distancing (this was typically 2 investigators).

If a participant developed a fever during the conduct of this study, they may have to undergo further testing as dictated by government advice at the time of the study. This may also require a period of isolation for the participant and their household. It was noted in the FLYBITE study that some study participants developed a subjective fever between visits, hence it was feasible in this study that this could develop.

In the unlikely event a fever develops during the first 48 hours after sand fly biting, in accordance with any government advice, self-isolation may necessary. However, if the fever

settles within 48 hours, it could be concluded that the fever is most likely due to sand fly biting and the participant can terminate any period of self-isolation. A significant fever in this situation was noted as >37.7C.

Participants were advised that if they develop a fever before attending for a study visit, they must not attend, including if the fever and any subsequent self-isolation period conflicted with a visit date. Participants were provided with a thermometer for use at home to substantiate any subjective fever that could develop and at the request of study investigators. In the event that a participant was unable to attend a visit due to any isolation period being necessary, then the visit could be deferred until such a time when it was feasible. At the discretion of the study investigators, it was possible that these visits could also be 'virtual visits', in addition to those already described in this section.

Participants could also be required to self-isolate for other reasons or symptoms as per any national guidance and were supported in doing so. This could include deferring visits and/or virtual visits as described above. A missed visit in the context of symptoms requiring self-isolation as per national guidance did not therefore constitute a deviation from this methodology.

Study participants were encouraged to undertake regular asymptomatic testing for SARS-CoV-2, if such facilities exist and are easily accessible (e.g. lateral flow testing), although this will not be mandatory. Lateral flow test kits were available from investigators if participants wished

	Pre-screening Days -90 to -14	**Visit 1** Screening Days -90 to -7	**Visit 2** Sand fly biting Day 0	**Visit 3** FU Day 4	**Visit 4** FU Day 14	**Visit 5** FU Day 28
Window	N/A	N/A	N/A	± 2 day	± 4 days	± 5 days
Information & discussion with the participant	X	X				
Consent		X	X			
History, Examination, Image documentation		X	X	X	X	X
Potential clinical diagnosis of CL, and excision biopsy						X
FBC, U+E, LFT, CRP,		X				X
HbA1c		X				
Total IgE, CD4 / CD8		X				
Blood borne virus screen (HIV, Hepatitis B/C)		X				
Leishmania serology		X				X
Peripheral blood mononuclear cells & immune monitoring		X			X	X
Serum β-HCG pregnancy test (females)		X				
Quality of Life questionnaires		X			X	X

Blood volume		60ml	0ml	0ml	30ml	50ml

Table 4.1: Schedule of Visits, Challenge Phase, out to 28 days post-challenge follow up

	Visit 6* Day 42	**Visit 7** Day 56	**Visit 8*** Day 70	**Visit 9** Day 98	**Visit 10*** Day 126	**Visit 11** Day 154
Window	+/- 7 days	+/-7 days	± 14 days	± 14 days	± 14 days	± 14 days
History, Examination, Image documentation	X	X	X	X	X	X
Potential clinical diagnosis of CL & excision biopsy	X	X	X	X	X	X
FBC, U+E, LFT, CRP		X		X		X
Leishmania serology		X		X		X
Peripheral blood mononuclear cells & immune monitoring		X		X		X
Quality of Life questionnaires		X		X		X
Blood volume	0ml	50ml	0ml	50ml	0ml	50ml

Table 4.2: Schedule of Visits, Challenge Phase, from day 42 to day 154 post-challenge follow up, pre-biopsy

*Scheduled as remote visits by video calling

	Visit 1 Day 0	**Visit 2** Day 10	**Visit 3** Day 30	**Visit 4** Day 60	**Visit 5** Day 90	**Visit 6** Day 180	**Visit 7** Day 360
Window	-	+/-3 days	± 3 days	± 7 days	± 14 days	± 21 days	± 21 days
History, Examination, Image documentation	X	X	X	X	X	X	X
Informed consent to biopsy / biopsies, treatment and follow up	X						
Excision biopsy / biopsies	X						
FBC, U+E, LFT, CRP	X*		X		X		
Leishmania serology	X*				X		
Peripheral blood mononuclear cells & immune monitoring	X*		X		X		
Quality of Life questionnaires	X	X	X	X	X	X	X
Blood volume	50ml	0ml	45ml	0ml	50ml	0ml	0ml

Table 4.3: Schedule of Visits, Treatment Phase, from day 0, the day of biopsy

*Depending on the transition from Table 1, Challenge Phase, these investigations will not be repeated if they have been previously performed in the preceding 7 days

4.2.17 Safety assessments

4.2.17.1 Definitions

Adverse Event (AE):
An AE was any untoward medical occurrence in a participant who has consented and is participating in a clinical study, including occurrences which are not necessarily caused by or related to that investigational item (in this case Sand Flies). An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease occurring during such a clinical study. Any adverse event noted prior to Day 0 (*Leishmania* challenge) did not need to be recorded.

Information on adverse events were collected on the day of challenge and at follow up visits as defined in the schedule. AEs were recorded by investigators through direct questioning, clinical examination, and diary card submissions. Events were graded according to the table in Appendix 1. In addition, systemic laboratory adverse events were collected through routine laboratory testing according to the schedule. These were recorded on the standard laboratory report. The information obtained were recorded in the participant's file.

Any results that were outside of the normal range for the laboratory at the local hospital but in the opinion of the investigators were not clinically significant and did not meet the criteria for being classed as an AE will not be classed as such. For events or results that were not specified in the table (Table 4.4 & Table 4.5), a decision was made by study investigators, as to whether the result was clinically significant. Only those events or results that were deemed clinically significant were classed as AE's.

Serious Adverse Event (SAE)
An SAE is an AE that results in any of the following outcomes, whether or not considered related to the sand fly bite.

- Death (i.e. results in death from any cause at any time)

- Life-threatening event (i.e. the participant was, in the view of a clinical investigator, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more serious form, might have caused death.
- Persistent or significant disability or incapacity (i.e. substantial disruption of one's ability to carry out normal life functions).
- Hospitalisation, regardless of length of stay, even if it is a precautionary measure for continued observation. Hospitalisation (including inpatient or outpatient hospitalization for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a serious AE.
- An important medical event (that may not cause death, be life threatening, or require hospitalisation) that may, based upon appropriate medical judgment, jeopardise the participant and/or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic reaction requiring intensive treatment in an emergency room or clinic, blood dyscrasias, or convulsions that do not result in inpatient hospitalization.
- Congenital anomaly or birth defect.

Solicited adverse events were expected. See Table 4.4 for further description of solicited adverse events.

4.2.17.2 Causality Assessment

For every AE, an assessment of the relationship of the event to the sand fly *Leishmania* challenge was undertaken by a Clinical Investigator. An interpretation of the causal relationship of the intervention to the AE in question was made using clinical judgment, based on the type of event; the relationship of the event to the time of challenge and alternative causes such as intercurrent or underlying illness and concomitant therapies (see Table 4.4 & Table 4.5)

Site	Adverse Event
Local (at the site of bite)	**Symptoms** Itch Pain / Discomfort **Signs** Erythema – grades 1,2,3,4 Swelling - grades 1,2,3,4 Blister - Vesicle < 5mm, or Bulla ≥5mm

Table 4.4: Solicited Adverse Events

0	No Relationship	Adverse events that can be clearly explained by extraneous causes and for which there is no plausible association with study product. Or adverse events for which there is no temporal relationship
1	Unlikely	Adverse events that may be temporally linked but which are more likely to be due to other causes than this study
2	Possible	Adverse event that could equally well be explained by the study or other causes, which are usually temporarily linked. Or of a similar pattern of response to that seen with other vector biting studies.
3	Probable	Adverse events that are temporarily linked and for which the study product is the more likely explanation than other causes. Or known pattern of response seen with other vector biting studies.

4	Definite	Adverse events that are temporarily linked and for which the study product is the most likely explanation. Or known pattern of response seen with other vector biting studies.

Table 4.5: Assessing the relationship of an AE to sand fly Leishmania challenge

4.2.17.3 Reporting Procedures for All Adverse Events

All adverse events occurring during the study observed by a Clinical Investigator or reported by the participant whether or not attributed to study procedure were reported in the CRF. The severity of clinical and laboratory adverse events were assessed according to the AE grading located in the appendix section of the protocol.

Solicited study events were recorded in the CRF but not reported to the sponsor as an AE (or recorded on an AE form) unless Grade 3 or higher (see Appendix Table 7.5: LEISH_Challenge solicited study adverse events grading).

4.2.17.4 Follow up

All adverse events that could result in a participant's withdrawal from the study or that could be present at the end of the study, were followed up until a satisfactory resolution occurred, or until a non-study related causality was assigned, or if otherwise deemed clinically stable by study clinical investigators.

4.2.17.5 Emergency Contact process

All study participants were given an emergency contact number to report and discuss any issues including AE's was a specific out-of-hours rota for clinical staff, with a single phone number for study participants to call. In addition to the emergency number, a phone number for the study office (during working hours) and my study investigator email address were provided, in addition to a generic study email address (see Figure 7.24).

4.2.17.6 Unexpected screening findings

Participants also underwent screening including a history, physical examination, and blood tests (as detailed above). If there were any abnormal or unexpected findings from these screenings, these were discussed with the participant by the clinical team. The participant was then referred to an appropriate medical speciality, or their GP if necessary (and the GP informed directly in either case). This did not necessarily exclude the participant from the study if this does not otherwise impact on the inclusion and exclusion criteria. The decision for ongoing participation were made in conjunction with the wider study team.

4.2.17.7 Reporting Procedures for Serious Adverse Events

An SAE report form and notification of the Sponsor was planned to take place within 24 hours of becoming aware any SAE. Any SAEs that were deemed to be causally linked to the administration of the protocol and unexpected were to be reported to the REC within 15 days of being made aware of the event. In addition, all non-solicited grade 3 & 4 AEs regardless of relationship were to be referred to the REC for consideration of whether they constitute the need for a halt to the study and/or protocol amendment.

4.2.17.8 Withdrawal of Participants

A participant had the right to withdraw from the study at any time and for any reason and were not obliged to give his or her reasons for doing so (including during the sand fly biting itself). A Clinical Investigator could withdraw the participant at any time in the interests of the participant's health and well-being. If withdrawal was due to an adverse event, appropriate follow-up visits or medical care were to be arranged until the adverse event resolved or stabilised. If a participant was considered to have failed the screening assessment or withdrew from the study at any time, either by choice or on the recommendation of clinical personnel, data and samples collected up to that point would remain available for analysis as part of the study, unless a participant withdraws consent for this.

Given the nature of the CHIM, the study intervention cannot be withdrawn after exposure to an infected sand fly bite. As such study participants were strongly encouraged to continue to attend follow-up visits. If a study participant decided to withdraw from any further follow-up, the sponsor was to be informed, and the participant was to be given contact details of the study investigators and where to seek medical attention if necessary. The participant's GP would also be contacted to inform them of events and the referral pathway if the participant later attended their GP with a suspected CL lesion. If a participant withdrew from the study, then later decided to return they were allowed to do so but only after agreement from the wider study team. The decision may be made by the study team to excise an early CL lesion in such participants to avoid an untreated CL lesion with loss to follow-up. It is noted that *Leishmania major* is not associated with dissemination and in most healthy immunocompetent subjects an untreated lesion will self-heal (Sghaier et al., 2022; McMahon-Pratt and Alexander, 2004).

If the participant was not enrolled into the biting portion of the study after being deemed ineligible, all screening data and samples collected up to that point would remain available for analysis as part of the study unless the participant withdrew consent for this. If there was deemed to be any information relevant for the participant's GP to be made aware at that stage, consent was taken to write to the GP. Consent for GP communication was taken at the initial screening visit.

If a participant wished to withdraw from the study, and then requested for their existing, un-analysed samples to be destroyed, the study investigators would initially discuss with the Sponsor what appropriate action should be taken. The final decision will then be made in conjunction with the participant as to which samples may be used in analyses if at all.

If they withdraw, then participants could be replaced in the study. A standby list of potential further participants was maintained.

4.2.17.9 Pregnancy Reporting

If a participant became pregnant during the 90 days following infected sand fly biting, they were followed up as other participants. The pregnancy was to be reported to the Sponsor in accordance. The treatment decision in this case were based on a discussion with the study clinical team and the participant.

Additional pregnancy testing was offered during the study if deemed necessary.

4.2.18 Clinical Criteria for Interruption or Discontinuation of *Leishmania* challenge:

"Discontinuation" is the permanent withholding of further sand fly exposure visits from all or some study groups in the study.

"Interruption of sand fly biting" is the temporary withholding of sand fly exposure if a serious adverse reaction is experienced during the study.

If any of the following occur, interruption or discontinuation of all further *Leishmania* challenge will take place and the Sponsor were notified within 24 hours.

(1) Death in any subject in which the cause of death is judged to be possibly, probably, or definitely related to sand fly bite exposure
(2) The occurrence in any subject of an anaphylactic reaction to sand fly bite
(3) If two or more participants experience an unexplained, unexpected grade 3 or 4 clinical or laboratory event (confirmed on attendance or repeat testing) that has not resolved within 72 hours and considered possibly, probably or definitely related to *Leishmania* challenge.

The Sponsor would then notify the wider study team / or the Sponsor would determine whether or not to call an unscheduled meeting to review the safety data, and whether or not to hold further sand fly biting visits until this has taken place.

If the study was halted or stopped for a reason involving risk to a participant's health or safety, then an Urgent Safety Measure was to be implemented. If the study was to be halted or stopped for any other reason the study team or Sponsors representative would notify the HRA not later than 15 days from the date of the halting of the study. In this situation, restarting the study would be a substantial amendment.

No more than 2 participants would undergo sand fly biting on any given day, and no participants would undergo sand fly biting simultaneously. Participants were scheduled to attend shortly after the biting visit (
Table 4.1, Table 4.2, Table 4.3). The development of any SAE or non-solicited grade 3 AE at the first post-biting review considered possibly, probably or definitely related to the biting procedure would result in a temporary halt and review of the sand fly biting parameters. Therefore, safety outcomes were reviewed 4 days after all biting procedures in real time for all subjects.

If an SAE or non-solicited grade 3 AE had been recorded the study investigators would review both the clinical event, and the biting parameters in terms of the number of sand flies, the species and length of exposure, with regard to progression of the study. If non-solicited Grade 3 / exaggerated reactions had been observed it would be likely that the Investigators would seek a protocol amendment to decrease the number of sand flies present within the biting chamber, or length of exposure to mitigate any exaggerated response. At the discretion of the clinical investigators it would be possible that a sufficient number of grade 2 AEs, would also result in such a halt to the study and consideration of a protocol amendment.

4.2.19 Reimbursement for Participants

Participants were compensated for their time and for the inconvenience at approximately these rates in relation to the following visits:

Screening visit – £20

Leishmania challenge visit £500

Follow-up visits, £40 per visit

Per completed diary card event - £5 (up to once weekly)

Per completed and participant-submitted photograph event - £5 (up to twice weekly)

Biopsy visit £500

Additional unscheduled visit £40

Additional minor surgery procedure £40

Small punch biopsy (4mm) diagnostic procedure £120

Cryotherapy treatment £40

Final visit £100

Focus Group visit £100

Total up to approximately £2500 depending on involvement for routine follow up.

Travel costs were reimbursed in addition in relation to expenses submitted.

Additional treatments and investigations were provided free of charge for any participants who might required them.

4.2.20 Public engagement project

I developed a parallel project supported by a small research grant from HIC-Vac (HIC-Vac Public Engagement Grant PE002, Imperial College reference PS3189) to produce a range of public-facing video to encourage engagement with CHIM studies. Given the increasing awareness of the importance of engagement of the public in research studies at all stages, this small bolt-on study allowed an insight into the mechanics of how a complex CHIM is run, as well access to researchers and participants.

4.2.20.1 Primary Objectives

To summarize the experience of controlled human infection studies, by previous participants in CHIM studies, and potential future participants in these studies

Outcome measures
Production of up to 5 short film(s) that describe the perception of CHIM studies amongst research participants

4.2.20.2 Secondary objectives

To share the findings and enhance the recognition of controlled human infection studies

Outcome measures
Sharing of short films via social media and websites

4.2.20.3 Inclusion & exclusion criteria

Inclusion criteria

- Aged 18 or over on the date of enrolment and able to comply with study procedures.

To include:

- Prior involvement with CHIM-related research (for media with respect to previous involvement)
- Enrolled in current/future CHIM-related research (for media with respect to current involvement)
- Researchers working on CHIM studies in the UK (for media with respect to researcher involvement)

Exclusion criteria

- Unwilling to give informed consent
- Unable to comply with study procedures

4.2.20.4 Study design & procedures

Participants were followed up at the point of intervention and shortly after, to share their experience by video recording. Study investigators and participants themselves, were supported to take part in the filming aspects.

Past members of the FLYBITE study were approached through existing links to encourage participants to share their story, experience and reflection through video recording.

Up to 5 short videos were produced which can be shared through social media. I made use of professional film makers and editors in order to produce a slick result, which resonates with the target audience.

The target were be 3-fold (as below)– This will predominantly be aimed at the 18 -50 year old viewer, i.e. those individuals who are likely to take part in CHIM studies. We will however aim to produce videos that are accessible to a wide audience, and therefore audiences such as school-age children, who have a passion for science may find the subject matter engaging.

4.2.20.5 Consent procedures

A consent form was filled by all contributors to ensure image rights are preserved. In addition to the consent form provided, further allied consent forms may be used for specific circumstances e.g. specific forms required by specific media companies.

Patients were identifiable in video, but only after consenting for this. No personal identifiable data were stored with external organisations, unless permission is given. Participants were not identifiable in any study report or publication, unless explicit permission was given.

4.3 Results

4.3.1 Participant demographics, screening and participant flow

One hundred and five participants were pre-screened and assessed for eligibility (see Figure 4.4: study CONSORT Flow diagram). Forty-seven participants declined further involvement and twenty-four were excluded with a further six placed on a waiting list. Twenty-eight participants were deemed potentially eligible and attended for in-person screening. Of these, twelve were excluded and two were placed on a waiting list. 14 participants were enrolled and progressed to the *Leishmania major*-infected sand fly biting study.

These enrolled participants were enrolled in two separate cohorts (Cohort 1 included 6 participants, Cohort 2 included 8 participants) as determined by the adaptive study design, with each cohort separated in time to allow for progress from the initial cohort to be analysed and then prospective changes to the study protocol to be made if needed, for example to address safety concerns, and to consider alternate vector. No participants withdrew from the study once enrolled. Sex at birth was recorded, and 66.7% and 50% female participants were enrolled in Cohorts 1 and 2 respectively. The age range of volunteers in Cohort 1 was 21 - 42 years (mean age 33.5) and Cohort 2 age range was 20 - 47 years (mean age 31.75). Important negative findings at screening included absence of clinically relevant systemic examination findings, no history of significant skin disease reported either by the participant or noted on examination, no history of anaphylaxis and no significant history of atopy.

See also appendix Figure 7.35 for images depicting lesion progress for all participants from study intervention until final visit, including post-biopsy. Images include standard digital imaging of the lesion from between 20-60cm distance with paired close-up digital dermoscopy where relevant.

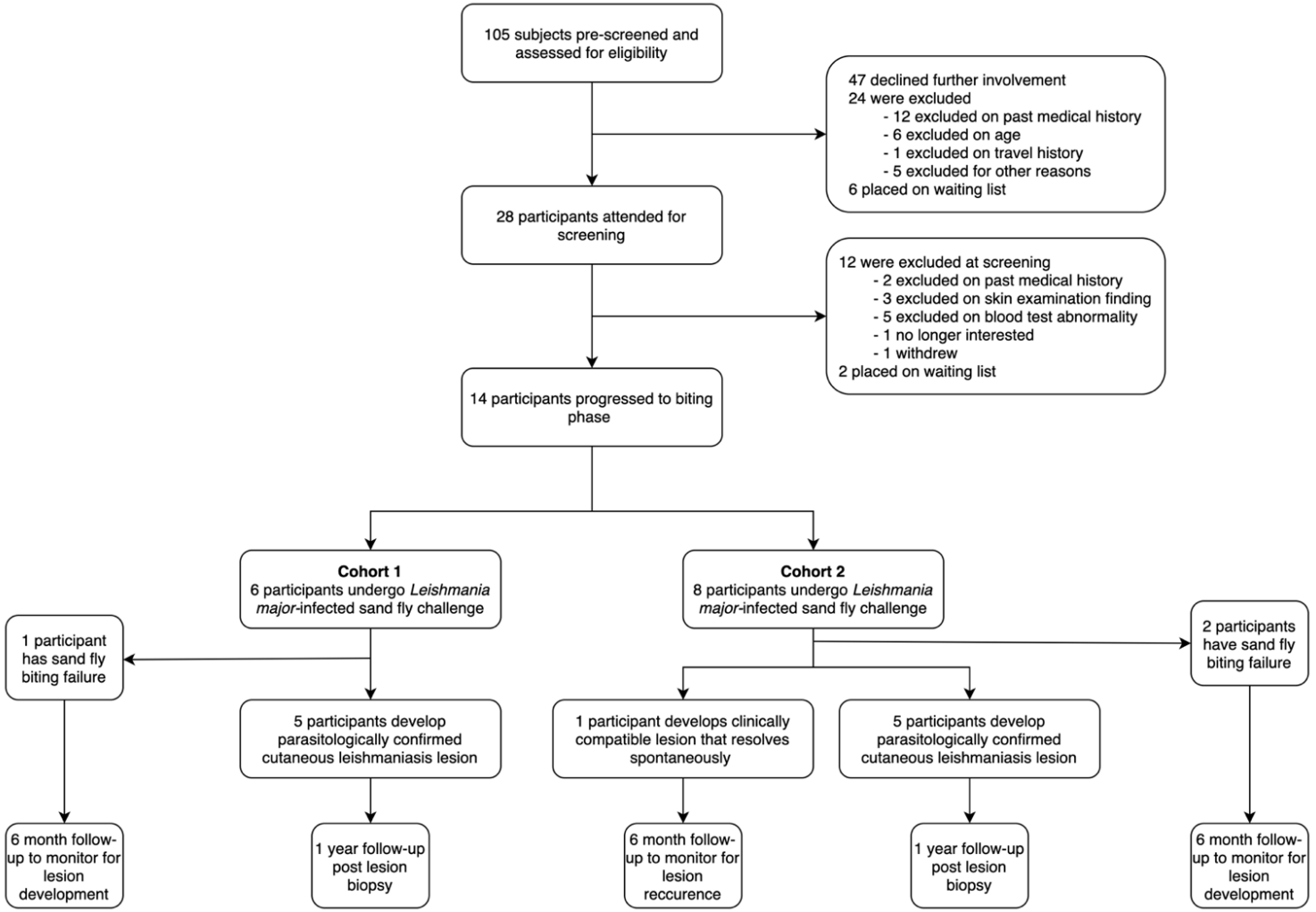

Figure 4.4: study CONSORT Flow diagram

Cohort 1: Screening commenced on 23rd November 2021 and 1st sand fly biting commenced on 24th January 2022.

Cohort 2: Screening commenced on 12th April 2022 and 1st sand fly biting commenced on 17th May 2022. All study biting visits were completed on 12th August 2022. All participants have completed up to at least 6 months post biting visits.

		Cohort 1 (n=6)	Cohort 2 (n=8)
Successful sand fly biting		5 (83.3%)	6 (75%)
Age, years		33.5 (7.40, 21-42)	31.75 (10.01, 20-47)
Sex at birth	Female	4 (66.7%)	4 (50%)
	Male	2 (33.3%)	4 (50%)
Medical history	Asthma	0 (0%)	1 (12.5%)
	Eczema	2 (33.3%)	2 (25%)
	Propensity to scarring	0 (0%)	0 (0%)
	Psoriasis	1 (16.7%)	0 (0%)
	Other respiratory conditions	0 (0%)	0 (0%)
	Cardiovascular conditions	1 (16.7%)	0 (0%)
Allergy history	Drug allergy	0 (0%)	0 (0%)
	Non-drug allergy	3 (50%)	3 (37.5%)
	History of anaphylaxis	0 (0%)	0 (0%)
Smoking	Current	2 (33.3%)	0 (0%)
	Former	2 (33.3%)	3 (37.5%)
Travel to areas where vectors for *Leishmania major* are endemic, in the past 10 years		0 (0%)	0 (0%)
COVID vaccination		6 (100%)	8 (100%)
Examination findings	Lymphadenopathy	0 (0%)	0 (0%)
	Other significant clinical finding	0 (0%)	0 (0%)
Skin examination at proposed bite site	Xeroderma	0 (0%)	0 (0%)
	Scaling	0 (0%)	0 (0%)
	Erythema	0 (0%)	0 (0%)
	Swelling	0 (0%)	0 (0%)
	Excoriation	0 (0%)	0 (0%)
	Ulceration	0 (0%)	0 (0%)
	Blistering	0 (0%)	0 (0%)
	Abnormal pigmentary change	0 (0%)	0 (0%)
	Minor scarring	1 (16.7%)	0 (0%)
	Significant scarring	0 (0%)	0 (0%)
General skin examination	Mild acne	0 (0%)	1 (12.5%)
	Other findings	0 (0%)	0 (0%)

Table 4.6: Baseline demographics and screening findings

This table shows participant findings at baseline obtained from clinical history, examination, and demographic data.

	CD4 count at screening			
	All participants (n=14/14)	Lesion recurrence (n=3/14)	Biting Failures (n=2/14)	Successful biting (n=12/14)
Mean CD4 ($x10^6$/L)	914.5714286	651.3333333	1080	887
Standard Deviation	238.1238316	70.76879413	340	203.8761879

Table 4.7: Baseline CD4 count

This table shows CD4 count, categorised by biting success.

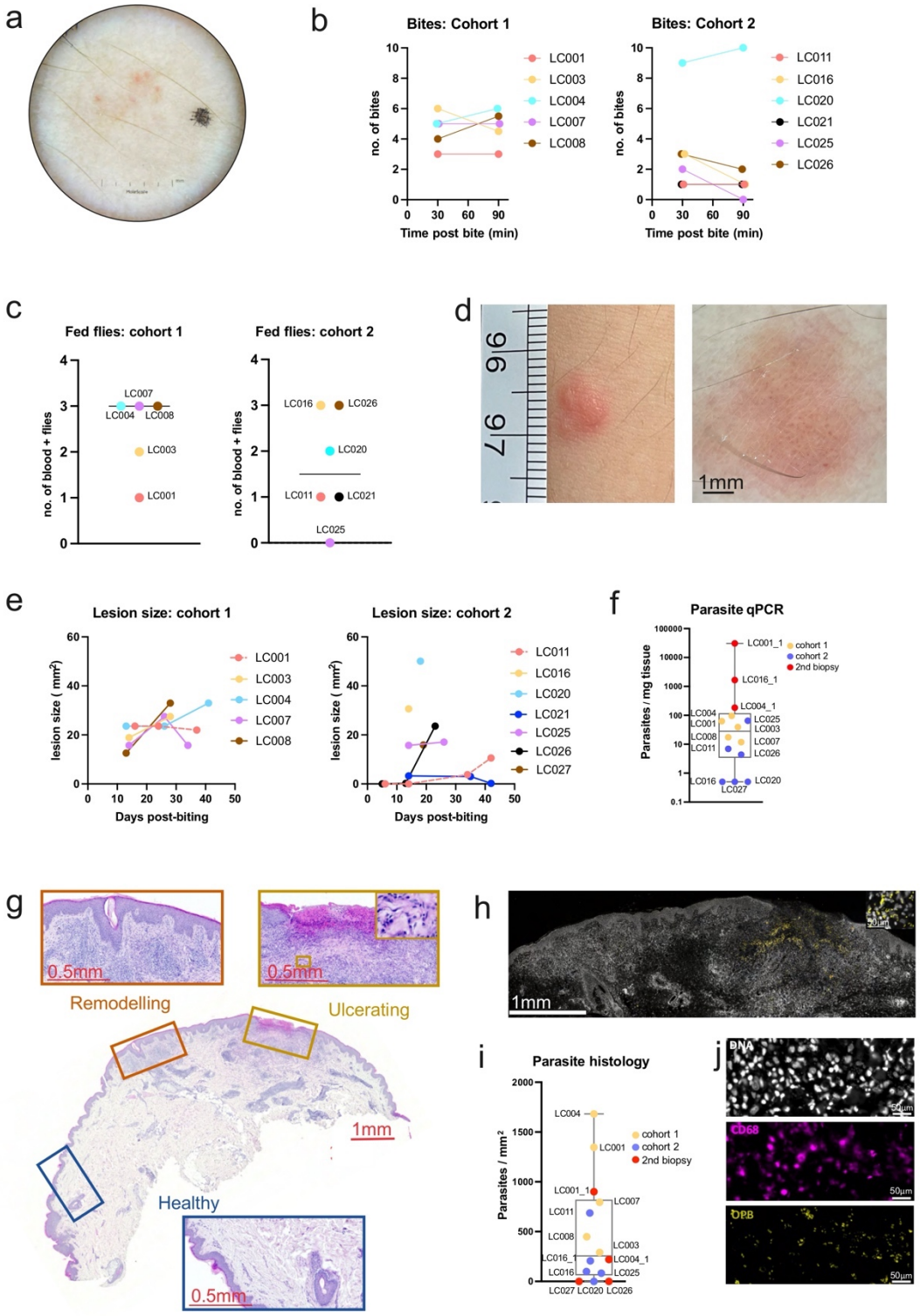

Figure 4.5: Lesion development and parasitological confirmation summary

Figure reproduced from (Parkash et al., 2024). **a)** Typical dermatoscopy findings immediately post-sand fly biting; **b)** number of observable sand fly bites in the post-biting period of observation (up to 90 minutes post-biting); **c)** number of sand flies demonstrating evidence of blood meal; **d)** Typical CL lesion development (LC004, 13 days post-bite). Paired

photography and dermoscopy images; **e)** lesion development with measured area (mm^2); **f)** Parasite load per mg biopsy tissue, qPCR. Box and whiskers plot with median, minimum and maximum values. All participants represented across both cohorts (n=14); **g)** Biopsy tissue from LC001, demonstrating typical H&E staining pattern. Areas of ulceration and remodelling at bite/lesion development site highlighted. Blue box indicates parasite demonsrates magnified area of parasite presence; **h)** immunohistochemistry for *Leishmania* Oligopeptidase B (yellow). Nuclear counterstain (white); **i)** Parasites per mm^2. Box and whiskers plot with median, minimum and maximum values. All participants represented across both cohorts (n=14). Quantitative morphometry; **j)** immunohistochemistry for *Leishmania* Oligopeptidase B (yellow) and CD68 (purple), demonstrating presence of parasite intracellularly. Additional nuclear staining (white), demonstrating characteristic nuclei and kinetoplast. Scale bar = 20µm.

(See also extended Figure 7.35: LEISH_Challenge picture summary for all participants)

4.3.2 Efficacy of sand fly biting study

Effectiveness of sand fly biting was determined by

(a) Participant-reported biting sensation during, and immediately after biting

(b) Suspected sand fly biting activity noted by clinical investigators (including video and photography during biting)

(c) Presence of bite compatible lesions by dermoscopy or photography immediately after biting

(d) Presence of a dilated abdomen in any of the sand flies in the biting chamber following withdrawal of biting chamber from participant, and subsequent dissection and microscopy of sand fly abdomen for presence of blood.

For both cohorts 5 sand flies were placed in each biting chamber and *P. duboscqi* sand flies were used throughout. Sand flies were placed initially on ice, which allows manipulation and handling to occur When placed into the sand fly biting chamber, some of the sand flies were observed to still be inactive as a result of contact with ice, however during sand fly biting, all sand flies demonstrated activity indicating that all participants had contact with 5 viable sand flies. During follow-up, there was no seroconversion demonstrated by use of rK39 rapid immunochromatographic test at specified timepoints (as per methods) until 3 months post-biopsy.

4.3.2.1 Cohort 1

All participants had a sand fly biting chamber attached with an aperture size of 6mm, that is the area through which sand flies can have contact with participant's skin for biting. There was no evidence of sand fly engorgement for any participant after direct visualisation of sand flies, immediately following biting, repeated by 2 observers. In each of these 6 participants, after dissection, 5/5 (100%) sand flies demonstrated evidence of *Leishmania* parasite presence in the mid-gut. In 5/6 (83.3%) participants, there was evidence of sand fly biting activity. All of the participants who had evidence of biting activity reported both a biting sensation and reported a pain of score of 1/10 (by visual analogue scoring) by the end of the 30 minutes biting chamber contact time. Immediately after removal of the biting

chamber the mean number of bites seen for these participants was 4.8 (range 3 – 6), which increased to a mean of 5 observable bites by 90 minutes following start of biting (range 3 – 6). 3/5 of these participants, who sustained a suspected sand fly bite, confirmed presence of an itching sensation (visual analogue scoring) during the sand fly biting period (range 1-4/10). In each of these 5 participants, observers noted sand fly activity which was consistent with sand fly probing behaviour. In each case, observer reported activity was noted earlier than subjective participant reported biting sensation. The sand fly behaviour was highly variable during the biting period, although the same behaviour was noted at investigator when suspecting sand fly probing, that is the sand flies were located outside the outer rim of the biting aperture, with the probiscis directed to towards the edge of the biting aperture (also mirrored by Cohort 2). In these suspected successfully bitten participants, upon sand fly dissection there was an average of 2.4 sand flies (range 1-3) that demonstrated evidence of blood meal. No sand fly demonstrated full abdominal engorgement, and instead partial filling with blood was noted. These 5 participants developed clinically compatible cutaneous leishmaniasis lesions. Biopsies took place at an average of 35.16 days following sand fly biting (range 28 – 42). All biopsies demonstrated evidence of *Leishmania* parasite by PCR.

In the participant who had suspected sand fly biting failure as per the aforementioned criteria, there was also absence of blood in the sand fly abdomen after culling and dissection, and absence of clinically compatible bite-lesions immediately after removal of the sand fly biting chamber, and in the follow-up period up to 150 minutes post start of biting. No lesion had developed by the day 28 follow-up visit. This participant subsequently developed a non-clinically compatible lesion close to the bite site at around day 35 post-biting and was 1mm x 1.5mm. This was papular, localised, without surrounding erythema and distinct from the bite lesions seen in the FLYBITE study. The lesion did not progress and remained static, with evidence of onset of resolution. Given the diagnostic challenge, the lesion was biopsied at day 48 post-biting, and no evidence of *Leishmania* parasite was detected by histological staining or PCR.

4.3.2.2 Cohort 2

Eight participants were included, expanding on the initial planned six participants by way of replacing participants experiencing sand fly biting failure in the study from within early in this cohort and cohort 1 (see section 4.5 Discussion). The average sand fly biting aperture for this cohort was 4.25mm (range 3 – 5 mm). 2/8 participants (25%) from Cohort 2 had suspected sand fly biting failure and were discussed later. With relevance to those six participants who underwent successful sand fly biting, all participants admitted to a subjective biting sensation by the end of the sand fly biting period. There was mixture of pain and itch reporting, with pain not universally admitted to by all participants. However in each case of successful sand fly biting, at least 1 of pain or itch was reported on a visual analogue score (range for pain: 0-2; range for itch 0-3 (/10)) by the end of the 30 minutes biting chamber contact time. Investigators noted sand fly probing behaviour for all these six participants (100%). 3/6 (50%) of these successfully bitten participants demonstrated partial engorgement on direct visualisation, with 1 sand fly being noted on each of the 3 occasions by 2 observers. This cohort had variable evidence of parasite burden within sand flies, on culling and dissection, with no correlation noted between successful biting or lesion development (Total Cohort 2 mean: 4, range 1-5).

Immediately after removal of the biting chamber the mean number of bites seen for these successful bitten participants was 3.16 (range 1 – 9). Observer reported activity was noted either simultaneously to that subjectively reported by participants, or before. In these six suspected successfully bitten participants, upon sand fly dissection there was an average of 1.67 sand flies (range 0-3) that demonstrated evidence of blood meal. For one participant who had successful biting including demonstrable bites, upon sand fly culling, there was no evidence of blood meal . No sand fly demonstrated full abdominal engorgement, and instead partial filling with blood was noted. These six participants developed clinically compatible cutaneous leishmaniasis lesions.

1/8 participants (12.5%) was deemed a sand fly biting failure and did not develop a clinically compatible cutaneous leishmaniasis lesion. 1 participant (12.5%) had suspected successful sand fly biting, and subsequently developed a clinically compatible lesion. However, this resolved rapidly and was not therefore amenable to biopsy and parasitological confirmation. 1 participant (12.5%) who had a suspected sand fly biting failure, went on to

subsequently develop a clinically compatible cutaneous leishmaniasis lesion which was parasitically-confirmed. The remaining 5/8 participants (62.5%) had both clinically compatible lesions and parasitologically-confirmed lesions. Where performed (6/8 participants), biopsies took place at an average of 23.67 days following sand fly biting (range 14 – 42). Lesion development was subjectively recorded closely, with a recommendation for daily recordings, for Cohort 2 using callipers given to each participant (see Figure 4.6, Figure 4.7 & Figure 4.8)

Cohort	Screening number	Days after biting that biopsy took place
1	LC001	37
	LC003	28
	LC004	41
	LC007	35
	LC008	28
	LC012	48
	LC011	42
2	LC016	14
	LC020	18
	LC021	No biopsy conducted
	LC023	No biopsy conducted
	LC025	26
	LC026	23
	LC027	19
	Mean	29.9
	Median	28.0
	Standard deviation	10.7
	Range	14-48

Table 4.8: Lesion biopsy day

This table shows the days from the sand biting day (Day 0), that the lesion biopsies took place. Mean, median, standard deviation and range does not include data from participants who did not undergo lesion biopsy.

4.3.2.3 Parasitological confirmation

All participants developed a lesion in the region of sand fly biting; 12/14 (85.71%) had a clinically compatible cutaneous leishmaniasis lesion, 2/14 (14.29%) had a non-clinically compatible lesion. Of those with clinically compatible lesions, 9/12 (75%) had parasitological confirmation using a combination of either qPCR and histological confirmation. 1/12 (8.33%) developed a clinically compatible lesion which self-resolved prior to biopsy. 1/12 (8.33%) was biopsied and qPCR and histology did not reveal evidence of *Leishmania* parasite presence, although there were no bite lesions seen immediately after biting and no sand fly evidence of blood meal. 1/12 (8.33%) had evidence of sand fly bite, and sand fly blood meal, with clinically compatible lesion, although qPCR did not reveal presence of parasites (See Table 4.9).

Therefore, attack rate was determined in several ways based on parasitological confirmation and clinically correlated lesion development. (See Table 4.9 for Summary of participant experience)

- Parasitological confirmation (i.e. qPCR+/-histology)
    - 9/14 (64.29%) [95% CI, 0.29, 1.22] total participants across both cohorts
    - 9/12 (75%) [95% CI, 0.34, 1.42] participants out of those who only developed clinically compatible lesions.
- Clinically compatible lesion development
    - 12/14 participants (85.71%) [95% CI, 0.44, 1.50] developed clinically compatible lesions out of total cohort.
    - 12/12 (100%) [95% CI, 0.52, 1.75] participants who were observed to have developed clinically compatible lesions.

Cohort	Participant	Evidence of sand fly bites	Evidence of sand fly blood meal	Clinically compatible lesion	Biopsy undertaken	qPCR confirmation	Histology confirmation	Recurrence
1	LC001	X	X	X	X	X	X	X
1	LC003	X	X	X	X	X	X	0
1	LC004	X	X	X	X	X	X	X
1	LC007	X	X	X	X	X	X	0
1	LC008	X	X	X	X	X	X	0
1	LC012	0	0	0	X	0	0	0
2	LC011	X	X	X	X	X	X	0
2	LC016	X	X	X	X	X	X	X
2	LC020	X	X	X	X	0	0	0
2	LC021	X	X	X	0	0	0	0
2	LC023	0	0	0	0	0	0	0
2	LC025	X	0	X	X	0	X	0
2	LC026	X	0	X	X	X	X	0
2	LC027	0	0	X	X	0	0	0

Table 4.9: Summary of participant experience

This table demonstrates the progress of all participants from both cohorts. This takes into account both histological and parasitological confirmation and feeds into attack rate calculations. Green cell with X indicates presence of the specified attribute, red cell with 0 indicates absence. Orange cell with X is used to denote episodes of disease recurrence.

Figure 4.6 and Figure 4.7 depict the progression in lesion development for Cohort 2. Due to the rapid lesion development noted in Cohort 1, Cohort 2 were encouraged to submit more regular measurements of subjectively recorded lesion diameter in 2 planes using callipers. Diameter is demonstrated, as well as an estimation of area based on an elliptical measurement ($A = \pi ab$), where a and b are the 2 diameter measurements recorded.

When a participant had a confirmed sand fly bite, in the majority of cases, the number of bites equalled or exceeded the number of sand flies that demonstrated evidence of blood feeding (by presence of blood) after dissection (See Figure 4.9).

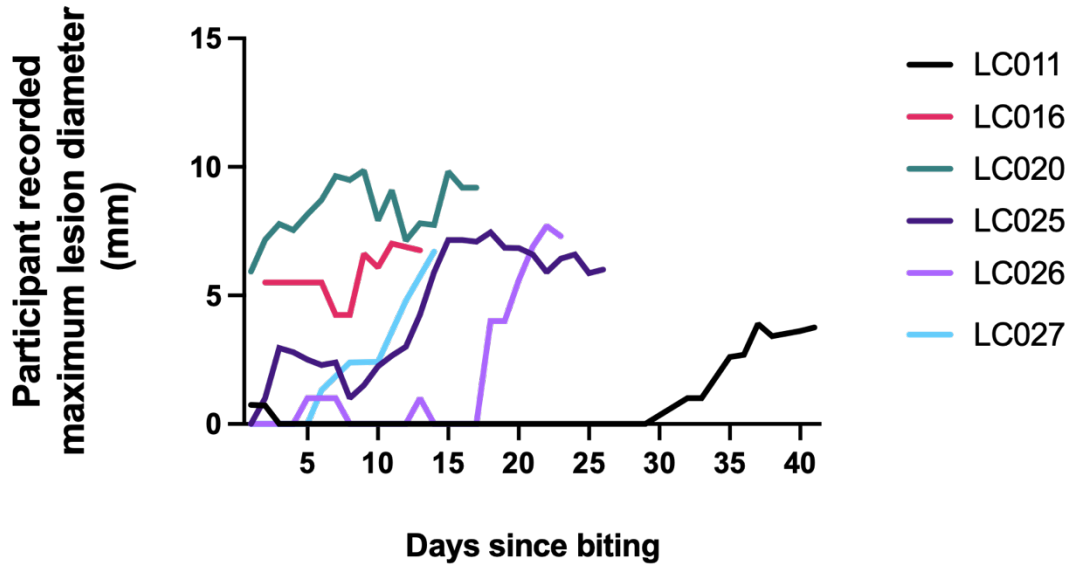

Figure 4.6: Cohort 2 participant recorded lesion diameter

For those participants who developed a clinically compatible CL lesion in Cohort 2, and subsequently underwent biopsy, maximal participant-recorded diameter of lesion by day has been shown until day of biopsy.

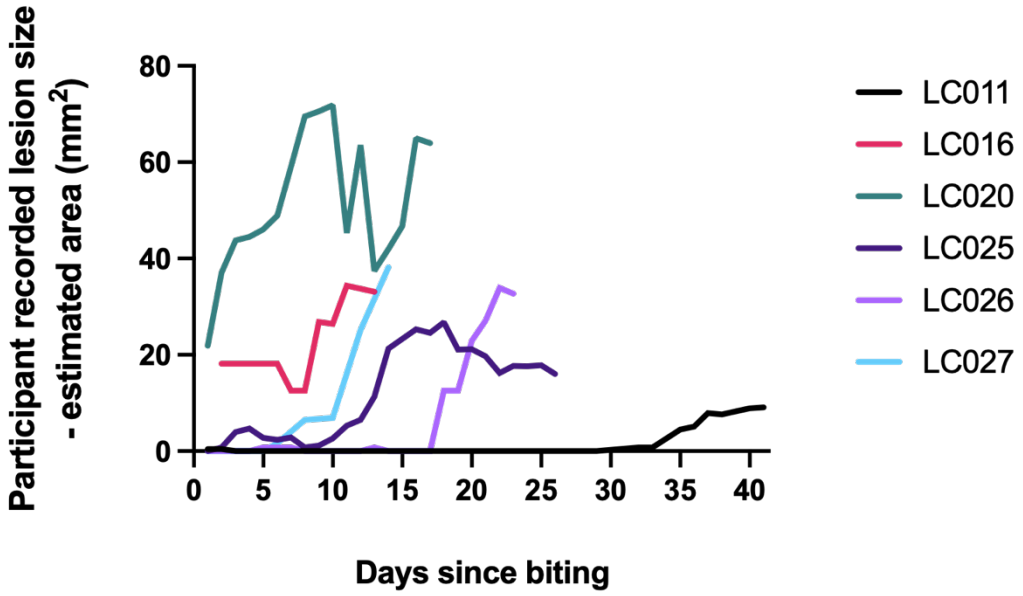

Figure 4.7: Cohort 2 participant recorded lesion estimated diameter

For those participants who developed a clinically compatible CL lesion in Cohort 2, and subsequently underwent biopsy, estimated diameter of lesion by day has been shown until day of biopsy. This was calculated by using two cross sectional diameters of the lesion, recorded by the participants, and calculating an estimated elliptical measurement ($A = \pi ab$).

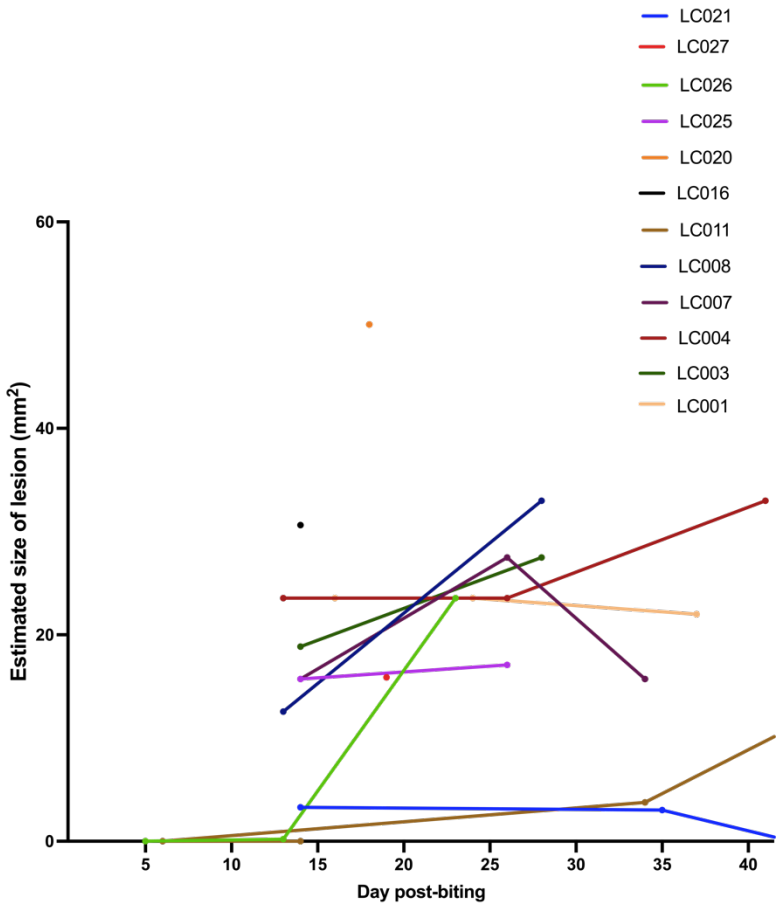

Figure 4.8: Clinician recorded lesion size.

Calculated using an estimated elliptical measurement (A = πab), after 2 clinician recorded diameters taken at planned visits and at biopsy visit. Participants included were those who had clinically compatible lesion development (n=12/14). LC021 had no biopsy, measurements are recorded until lesion resolution. LC016, LC020 & LC027 have only 1 measurement (day of biopsy) due to rapid lesion development.

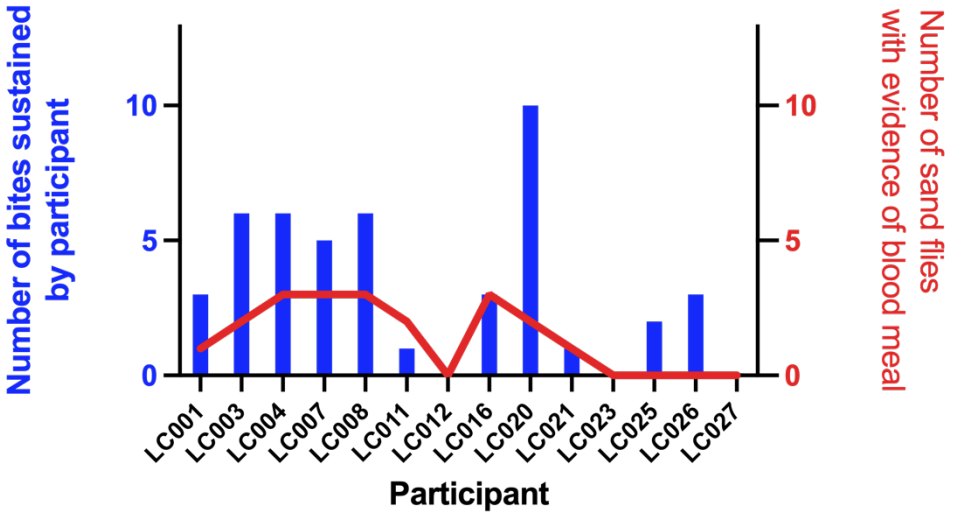

Figure 4.9: Graph showing sand fly bites sustained vs sand flies with evidence of blood meal

The number of bites sustained by each participant is shown as a blue bar, and the number of sand flies that demonstrated evidence of taking a blood meal is shown as a red line.

		Cohort 1					
		LC001	LC003	LC004	LC007	LC008	LC012
Sand fly species		*P. duboscqi*	*P. duboscqi*	*P. duboscqi*	*P. duboscqi*	*P. duboscqi*	*P. duboscqi*
Sand fly bite site (Right vs Left medial forearm)		Right	Left	Left	Left	Left	Left
Sand fly biting aperture (mm)		6	6	6	6	6	6
Number of sand flies in biting chamber		5	5	5	5	5	5
At 1st biting sensation	*Time of 1st biting sensation by participant (mm:ss)*	6:00	10:00	2:00	0:20	2:00	NA
	Time of 1st biting activity by investigator (mm:ss)	2:30	8:00	0:30	0:10	1:00	17:00
	Nature of 1st biting activity	Sand fly probing	Sand fly probing	Sand fly probing	Sand fly probing	Sand fly probing	Other
At 1st biting sensation - Visual Analogue score (/10)	*Pain/discomfort*	1	1	0	1	1	0
	Itch	0	0	0	0	1	0
Biting sensation reported by participant by 15 minutes after start of sand fly biting		Yes	Yes	Yes	Yes	Yes	No
15 minutes after start of biting - Visual Analogue score (/10)	*Pain/discomfort*	1	1	0	0	1	0
	Itch	1	0	0	1	4	0
30 minutes after start of biting - Visual Analogue score (/10)	*Pain/discomfort*	0	0	1	0	0	0
	Itch	1	0	0	1	2	0
Biting sensation reported by participant by 30 minutes after start of sand fly biting		Yes	Yes	Yes	Yes	Yes	No
Sand flies demonstrating engorgement (reviewer 1)		0	0	0	0	0	0
Sand flies demonstrating engorgement (reviewer 2)		0	0	0	0	0	0
If sand fly engorgement noted	*Number of sand flies showing evidence of feeding after microscopy*	1	2	3	3	3	0
	Partially filled / Engorged	Partially filled	Partially filled	Partially filled	Partially filled	Partially filled	Partially filled
Number of sand flies with evidence of parasites after culling		5	5	5	5	5	5
Bite site examination (30 mins, Reviewer 1)	*Dermatoscopic evidence of biting*	Yes	Yes	Yes	Yes	Yes	No
	Number of bites (dermatoscopy)	3	6	5	5	4	0
	Dermatoscopic evidence of erythema (graded scored 0-3)	2	1	1	1	2	0
	Dermatoscopic evidence of swelling (graded score 0-3)	0	0	0	0	0	0
	Dermatoscopic evidence of blistering (graded score 0-3)	0	0	0	0	0	0
Bite site examination (30 mins, Reviewer 2)	*Dermatoscopic evidence of biting*	Yes	Yes	Yes	Yes	Yes	No
	Number of bites (dermatoscopy)	3	6	5	5	4	0
	Dermatoscopic evidence of erythema (graded scored 0-3)	2	1	1	1	2	0
	Dermatoscopic evidence of swelling (graded score 0-3)	0	0	0	0	0	0
	Dermatoscopic evidence of blistering (graded score 0-3)	0	0	0	0	0	0
90 minutes after start of biting - Visual Analogue score (0/10)	*Pain/discomfort*	0	0	0	0	0	0
	Itch	0	0	0	0	0	0
Bite site examination (90 mins, Reviewer 1)	*Dermatoscopic evidence of biting*	Yes	Yes	Yes	Yes	Yes	No
	Number of bites (dermatoscopy)	3	5	6	5	6	0
	Dermatoscopic evidence of erythema (graded scored 0-3)	1	1	1	1	1	0
	Dermatoscopic evidence of swelling (graded score 0-3)	0	0	0	0	0	0
	Dermatoscopic evidence of blistering (graded score 0-3)	0	0	0	0	0	0
150 minutes after start of biting - Visual Analogue score (/10)	*Pain/discomfort*	0	0	0	0	0	0
	Itch	0	0	0	0	0	0
AE reporting by participant by 150 minutes post start of biting		No	No	No	No	No	No

Table 4.10: Summary of sand fly biting day experience – Cohort 1

		Cohort 2							
		LC011	LC016	LC020	LC021	LC023	LC025	LC026	LC027
Sand fly species		*P. duboscqi*	*P. duboscqi*	*P. duboscqi*	*P. duboscqi*	*P. duboscqi*	*P. duboscqi*	*P. duboscqi*	*P. duboscqi*
Sand fly bite site (Right vs Left medial forearm)		Right	Left	Left	Left	Left	Left	Left	Left
Sand fly biting aperture (mm)		4	3	5	4	4	5	4	5
Number of sand flies in biting chamber		5	5	5	5	5	5	5	5
At 1st biting sensation	*Time of 1st biting sensation by participant (mm:ss)*	29:00	3:00	8:00	13:00	NA	8:00	6:00	NA
	Time of 1st biting activity by investigator (mm:ss)	29:00	2:00	8:00	13:00	NA	4:00	1:00	NA
	Nature of 1st biting activity	Sand fly probing	Sand fly probing	Sand fly probing	Sand fly probing	Other	Sand fly probing	Sand fly probing	Nil
At 1st biting sensation - Visual Analogue score (/10)	*Pain/discomfort*	1	1	2	0	0	1	0	0
	Itch	0	2	2	3	0	0	1	0
Biting sensation reported by participant by 15 minutes after start of sand fly biting		Yes	Yes	Yes	Yes	No	Yes	Yes	No
15 minutes after start of biting - Visual Analogue score (/10)	*Pain/discomfort*	0	1	1	0	0	0	0	0
	Itch	0	3	2	2	0	0	0	0
30 minutes after start of biting - Visual Analogue score (/10)	*Pain/discomfort*	0	1	1	0	0	0	0	1
	Itch	0	1	1	0	0	0	0	0
Biting sensation reported by participant by 30 minutes after start of sand fly biting		Yes	Yes	Yes	Yes	No	Yes	Yes	Not sure
Sand flies demonstrating engorgement (reviewer 1)		0	1	0	1	0	0	1	0
Sand flies demonstrating engorgement (reviewer 2)		0	1	0	1	0	0	1	0
If sand fly engorgement noted	*Number of sand flies showing evidence of feeding after microscopy*	1	3	2	1	0	0	3	0
	Partially filled / Engorged	Partially filled	Partially filled	Partially filled	Partially filled	Partially filled	Partially filled	Partially filled	Partially filled
Number of sand flies with evidence of parasites after culling		1	5	5	2	5	5	3	5
Bite site examination (30 mins, Reviewer 1)	*Dermatoscopic evidence of biting*	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
	Number of bites (dermatoscopy)	1	3	9	1	0	2	3	0
	Dermatoscopic evidence of erythema (graded scored 0-3)	0	1	1	0	0	2	1	0
	Dermatoscopic evidence of swelling (graded score 0-3)	0	0	0	0	0	0	0	0
	Dermatoscopic evidence of blistering (graded score 0-3)	0	0	0	0	0	0	0	0
Bite site examination (30 mins, Reviewer 2)	*Dermatoscopic evidence of biting*	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
	Number of bites (dermatoscopy)	1	3	9	1	0	2	3	0
	Dermatoscopic evidence of erythema (graded scored 0-3)	0	1	1	1	0	2	1	0
	Dermatoscopic evidence of swelling (graded score 0-3)	0	0	0	0	0	0	0	0
	Dermatoscopic evidence of blistering (graded score 0-3)	0	0	0	0	0	0	0	0
90 minutes after start of biting - Visual Analogue score (0/10)	*Pain/discomfort*	0	0	0	0	0	0	0	0
	Itch	0	0	0	0	0	0	0	0
Bite site examination (90 mins, Reviewer 1)	*Dermatoscopic evidence of biting*	Yes	Yes	Yes	Yes	No	No	Yes	No
	Number of bites (dermatoscopy)	1	1	10	1	0	0	2	0
	Dermatoscopic evidence of erythema (graded scored 0-3)	1	1	1	0	0	0	1	0
	Dermatoscopic evidence of swelling (graded score 0-3)	0	0	0	0	0	0	0	0
	Dermatoscopic evidence of blistering (graded score 0-3)	0	0	0	0	0	0	0	0
150 minutes after start of biting - Visual Analogue score (/10)	*Pain/discomfort*	0	0	0	0	0	0	0	0
	Itch	0	0	0	0	0	0	0	0
AE reporting by participant by 150 minutes post start of biting		No	No	No	No	No	No	No	No

Table 4.11: Summary of sand fly biting day experience – Cohort 2

4.3.3 Safety of sand fly biting study

There were no SAEs or SUSARs during the study, and no grade 3 AEs. Safety data was recorded based on clinical findings during follow-up and clinical review of participant-submitted pictures and blood tests. Participants were also given the opportunity to provide diary card data (uploaded electronically) which was encouraged daily during the pre-biopsy phase and then weekly to monthly thereafter, until around 6 months post-biopsy, when it was encouraged 3 monthly. Compliance was generally good, although 2 participants had large gaps against the above schedule despite several e-reminders. Diary card data included visual analogue scoring for a pre-determined list of signs and symptoms based on expected solicited findings, and experience from FLYBITE of common sand fly bite-associated solicited events. Photo data from the lesion site was uploaded (the 2nd cohort were provided with callipers to accurately report size), quality of life measures at defined time points (DLQI and GAD-7), and opportunity to add medical history and other information as needed.

Participant clinical observations (heart rate, temperature, blood pressure and respiratory rate) were recorded prior to sand fly biting, immediately after removal of sand fly biting chamber, and then at 30-minute intervals until 150 minutes post-sand fly biting. No participant had any abnormal readings during the biting day. Additionally, these recordings were performed at each in-person visit, and no persistently abnormal or clinically relevant values were detected. Data on AEs was recorded and graded according to the methods in this chapter. Solicited effects of the study were not reported as AEs unless grade 3 or higher as per the methods, although were captured using visual analogue scoring by diary card. 36 separate AEs were recorded, of which 23 were reported as either 'Unlikely' or 'No relationship' in relation to the study, and these were all grade 1. 13 AEs were reported as causally either 'Definite' or 'Probable' in relation to the study (see Table 4.12& Table 4.13). There were no grade 3 or higher AEs reported during the study. 3/14 participants (21.4%) reported a recurrence of leishmaniasis following the primary biopsy (see Recurrence of leishmaniasis section). 6/14 participants (42.86%) reported hypertrophic scarring, which responded to application of a topical silicon-based scar cream. This was the most reported study associated event. 2/14 participants (14.2%) reported a post-biopsy wound infection which responded to a short course of oral antibiotics (Flucloxacillin). One of the participants

who developed a wound infection, had a bacterial skin swab of the visible exudate taken which was cultured and grew methicillin-sensitive *Staphylococcus aureus*. The other participant did not develop sufficient exudate that would be amenable to microbial swab and subsequent culture.

AE number	Event	Grading	Relationship to study	Treatment required
2	Recurrence of Leishmaniasis	G2	Definite	Yes
3	Hypertrophic scar	G1	Definite	Yes
4	Exudate from scar site	G1	Probable	No
6	Wound site infection	G2	Probable	Yes
8	Inflamatory scarring	G2	Probable	Yes
9	Hypertrophic scar	G2	Definite	Yes
12	Wound site infection	G2	Probable	Yes
13	Hypertrophic scar	G2	Definite	Yes
14	Recurrence of Leishmaniasis	G2	Definite	Yes
17	Hypertrophic scar	G1	Definite	Yes
22	Recurrence of Leishmaniasis	G2	Definite	Yes
23	Hypertrophic scar	G1	Definite	Yes
25	Hypertrophic scar	G1	Definite	Yes

Table 4.12: Study-related unsolicited AEs.

The most reported study-unrelated AEs were upper respiratory tract infections, with 5 participants reporting these, followed by COVID-19 infection with 3 participants reporting these. One participant had persistence and increase in pre-existing thrombophilia, which was investigated by NHS services, without long-term follow-up required. No other blood tests abnormalities were reported as AEs as per protocol definition.

AE number	Event Name	Grade	Relationship to study	Medication/treatment for AE	SAE
1	Folliculitis	G1	Unlikely	No	No
6	Upper respiratory tract infection	G1	Unlikely	No	No
9	Hay fever	G1	Unlikely	Yes	No
10	COVID positive	G1	Unlikely	No	No
14	Recurrent orolabial herpes simplex virus	G1	Unlikely	Yes	No
15	Upper respiratory tract infection	G1	Unlikely	No	No
17	COVID positive	G1	Unlikely	No	No
18	Bacterial throat infection	G1	Unlikely	Yes	No
19	Dental infection	G1	Unlikely	Yes	No
20	Acanthosis nigricans	G1	Unlikely	No	No
23	Persistence of pre-existing thrombophilia	G2	Unlikely	No	No
25	Urinary tract infection	G1	Unlikely	Yes	No
26	Migraine	G1	Unlikely	No	No
27	Abdominal bloating	G1	Unlikely	Yes	No
28	Toothache	G1	Unlikely	Yes	No
29	Hay fever	G1	Unlikely	Yes	No
30	Upper respiratory tract infection	G1	Unlikely	No	No
31	Upper respiratory tract infection	G1	Unlikely	No	No
32	COVID positive	G1	Unlikely	No	No
33	Migraine	G1	Unlikely	No	No
34	New mole	G1	Unlikely	No	No
35	Upper respiratory tract infection with fever with rash	G1	Unlikely	Yes	No
36	Fall	G1	No relationship	No	No

Table 4.13: Study-unrelated solicited AEs

Solicited data were recorded by use of diary card Visual Analogue Score data. This was prospectively recorded, and subjective data given by participants. Each individual score was out of a total of 10 for 8 separate possible adverse effects. Summed data for each participant for each of the prospectively recorded elements is shown in Figure 4.13. The maximum possible VAS score was 80, with the maximum actual summed VAS being 9/80 until either the day of primary biopsy or lesion resolution.

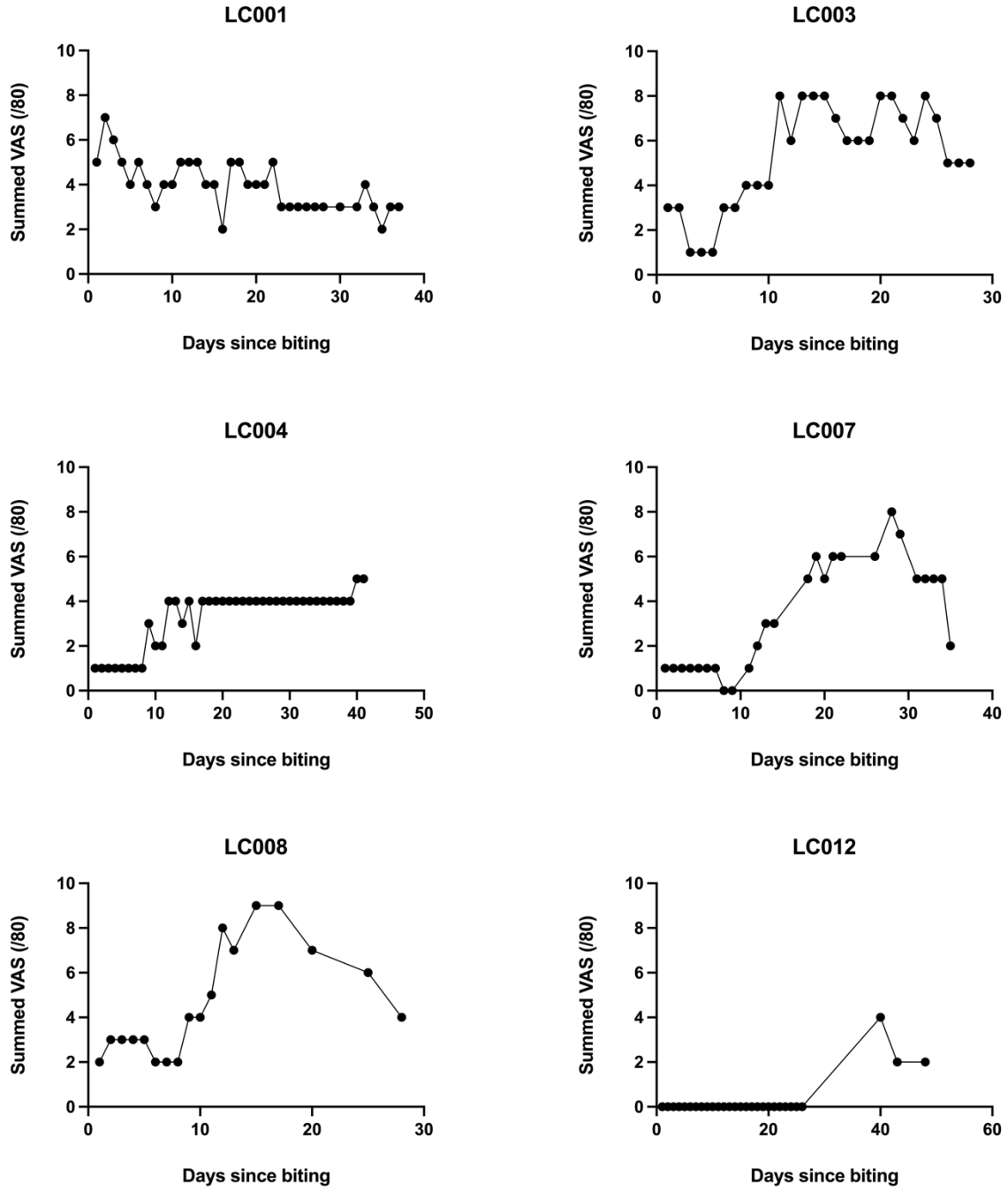

Figure 4.10: Summed Visual Analogue Score (VAS) for each participant – Cohort 1.

Eight adverse events were prospectively recorded by each participant at each visit on a visual analogue scale of 0–10 (see Methods). Data are presented as sum of all scores (out of 80) for each participant. Data shown is until day of biopsy, including for LC012 – a suspected sand fly biting failure with lesion non-clinically compatible with CL.

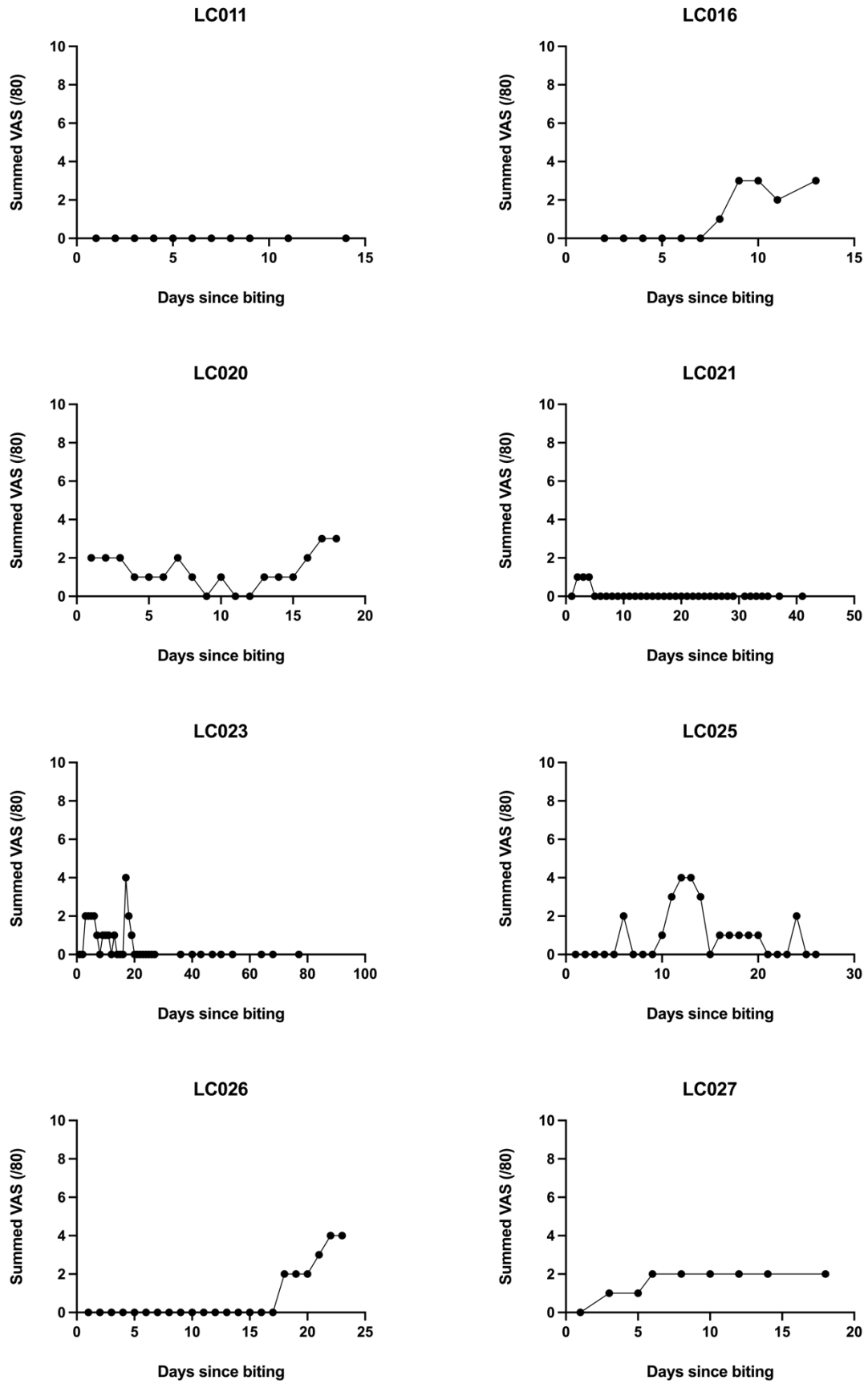

Figure 4.11: Summed Visual Analogue Score (VAS) for each participant – Cohort 2.

Eight adverse events were prospectively recorded by each participant at each visit on a visual analogue scale of 0–10 (see Methods). Data are presented as sum of all scores (out of 80) for each participant Data shown is until day of biopsy or disappearance of lesion, including for biting failures. For LC021 (suspected CL lesion self- resolving lesion) and LC023 (suspected biting failure) data is shown until disappearance of any local lesion, even if not clinically compatible with CL.

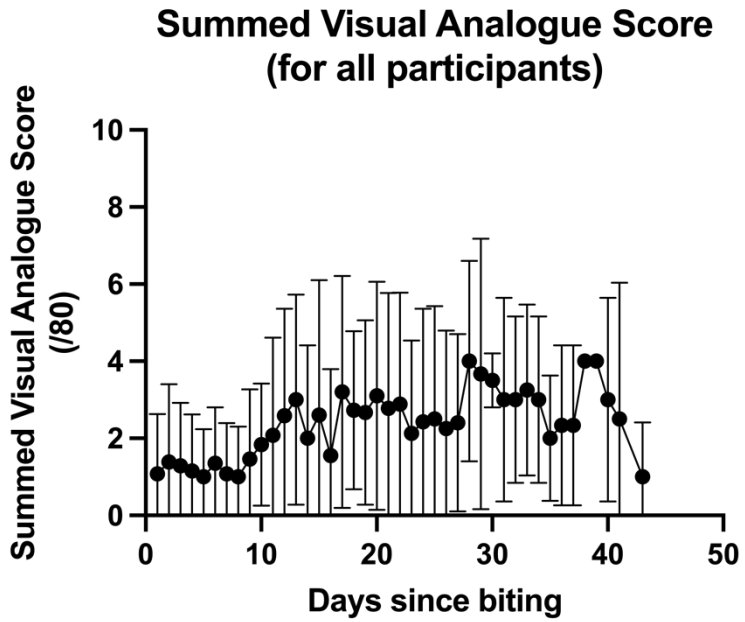

Figure 4.12: Graph showing aggregated summed VAS scores.

Visual analogue scores are given out of a total of 80. Data is shown for 14/14 participants until lesion biopsy or until lesion resolution. Black circles represent the mean score, and bars represent standard deviation.

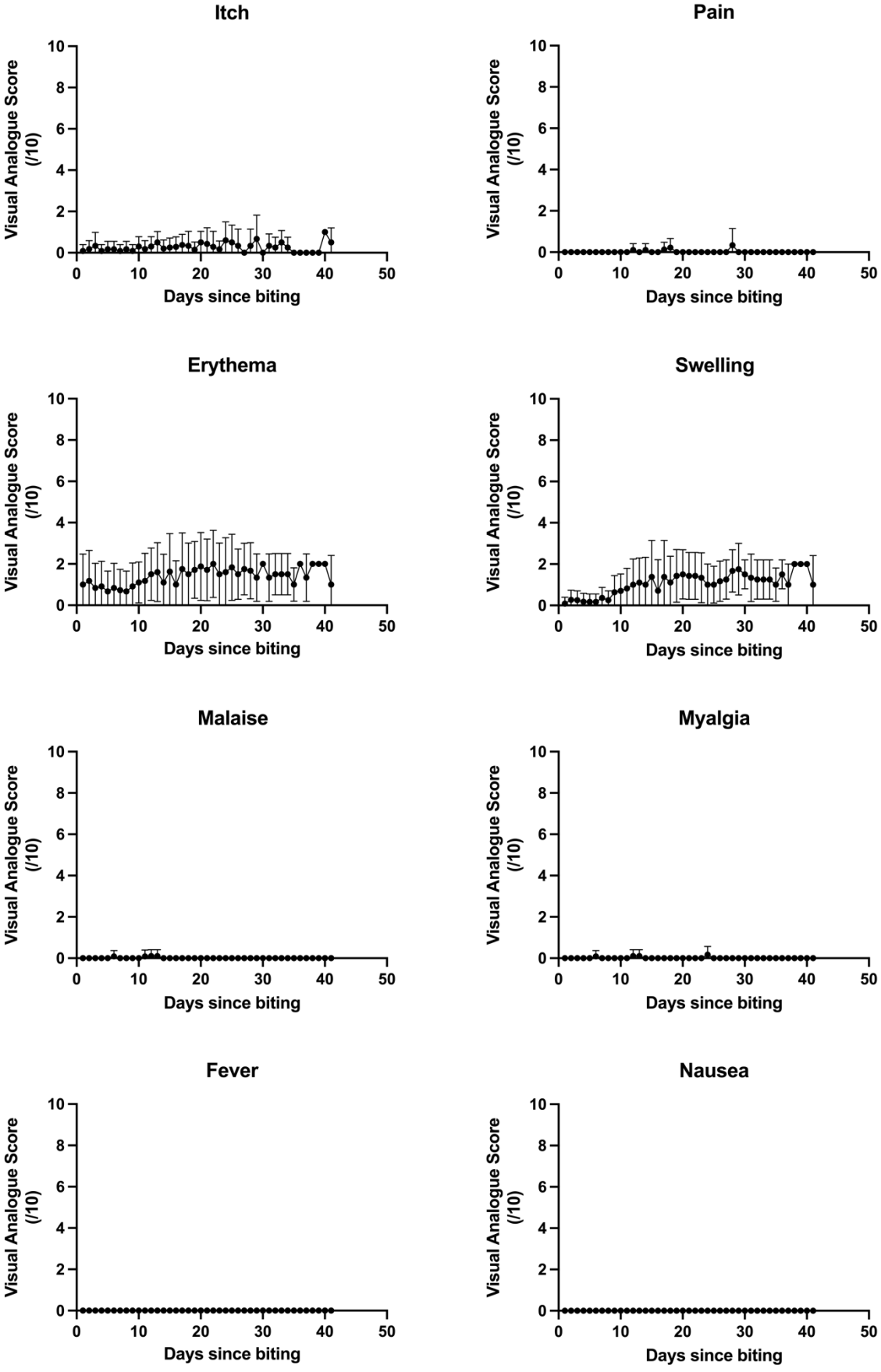

Figure 4.13: Graphs showing VAS scores by each solicited event.

For each solicited event, participants electronically uploaded a prospectively recorded Visual Analogue Score. Data shown includes participants who were had clinically compatible CL lesions (n=12/14). Data is given either until date of primary biopsy or until lesion resolution. Black circles represent mean scores, and bars represent standard deviation.

Solicited events are depicted in Figure 4.13, up to the point of biopsy or lesion resolution in those with clinically compatible CL lesions. Solicited events at the bite site are most common with erythema and swelling predominating. Pain scores remained low, with the maximum pain being 2/10 after biting until primary lesion biopsy or resolution.

Following primary lesion biopsy or lesion resolution, VAS scores continued to be recorded throughout follow-up. To-date at least 1 year of follow-up VAS data has been collected for Cohort 1 and 9 months for Cohort 2. Maximal values for each domain and for Summed VAS are given in Table 4.14. VAS scores went up after lesion biopsy partly reflecting impact of biopsy and treatment, but also length of follow-up and potentially confounding factors such as other co-existing illness. For example, the maximum VAS for Cohort 2 was 19/80, which coincided with a new diagnosis of symptomatic COVID-19, with the next highest summed VAS for Cohort 2 being 8/80. The peak pain score for any participant throughout the study was 4/10, which occurred during the post-biopsy follow-up phase.

	Itch (/10)	Pain (0/10)	Erythema (0/10)	Swelling (0/10)	Malaise (0/10)	Myalgia (0/10)	Fever (0/10)	Nausea (/10)	Maximum Summed VAS (/80)
Cohort 1	2	4	6	5	3	2	0	2	11
Cohort 2	5	4	4	3	6	6	7	0	19
Overall	5	4	6	5	6	6	7	2	19

Table 4.14: Maximum VAS scores throughout the study.

Maximum visual analogue scores are given for each solicited event (out of 10), and a maximum summed VAS score is given (out of 80), including post-biopsy and/or lesion resolution until at 1 year (Cohort 1) or at least 9 months (Cohort 2) follow-up.

Blood tests results for all participants are shown in Figure 4.14 & Figure 4.15. Participants were followed up in 2 phases: post-sand fly biting and post-biopsy. Participants were due to have bloods drawn prior to biopsy or lesion resolution. However due to rapid lesion development, participants did not have bloods taken on early study visits and instead attended for a biopsy. Some participants did not have bloods taken on the day of biopsy as planned, due to issues with venous access and logistical issues. As such, and to give a clearer indication of any significant changes from screening results, I have represented the screening/baseline values against peak values (to include the whole post-biting period from lesion development to 3 months post-biopsy, where relevant). The median and interquartile ranges remain within the normal laboratory values.

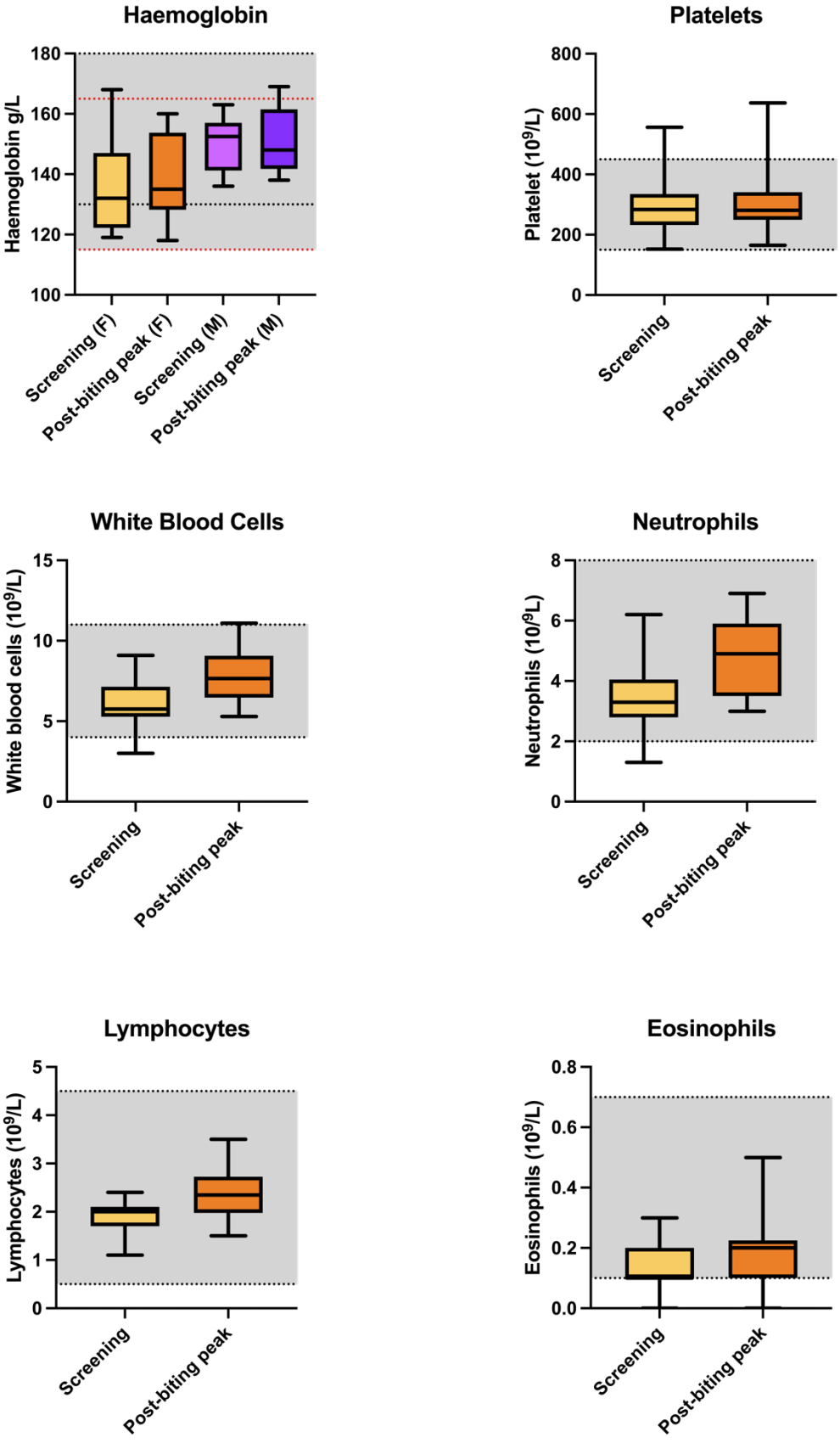

Figure 4.14: Participant blood tests results showing full blood count.

Box and whiskers plot for all participants, with screening results compared to post-biting peak results taken up to 90 days post-biopsy. Dotted black line with grey shading indicates normal laboratory range for each test. Where range differs for male and female participants, separate box and whisker plots are given for each sex, male range is denoted by the dotted black line and female range is denoted by dotted red line. Where indicated F denotes female participant results and M denotes male participant results.

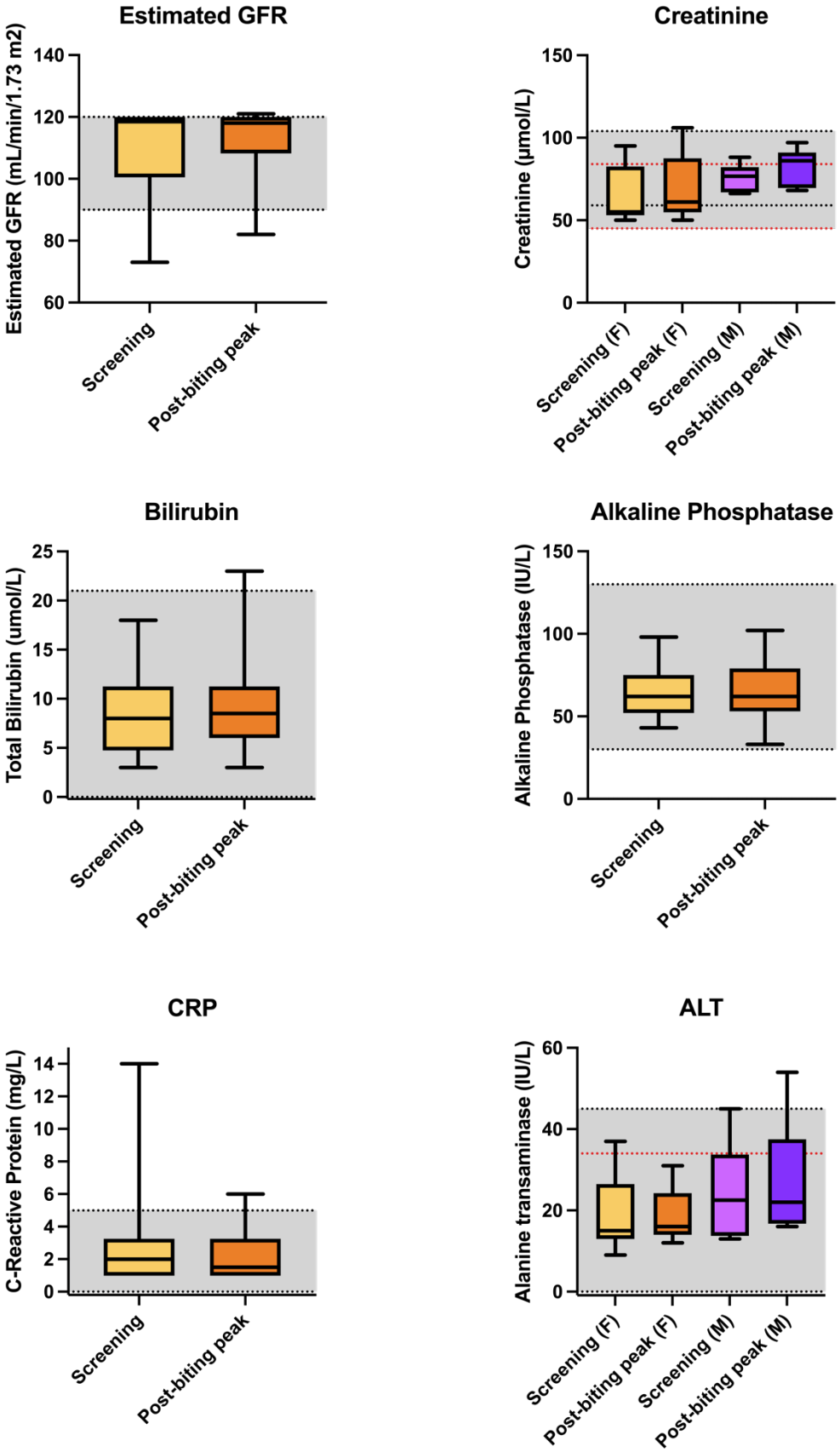

Figure 4.15: Participant blood tests results showing clinical chemistry tests.

Box and whiskers plot for all participants, with screening results compared to post-biting peak results taken up to 90 days post-biopsy. Dotted black line with grey shading indicates normal laboratory range for each test. Where range differs for male and female participants, separate box and whisker plots are given for each sex, male range is denoted by the dotted black line and female range is denoted by dotted red line. Where indicated F denotes female participant results and M denotes male participant results.

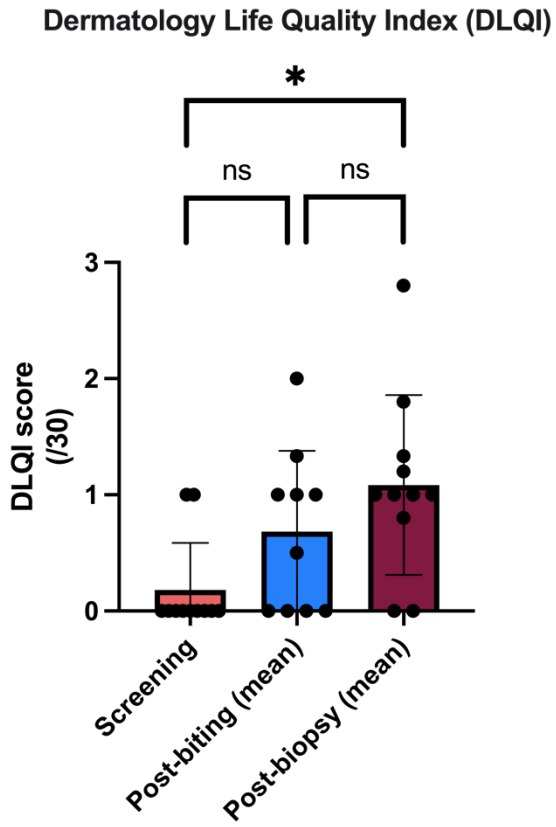

Figure 4.16: Scatter plot showing mean Dermatology Life Quality Index scores.

At each time period, mean scores are given for all participants who underwent biopsy and had successful biting (n=11/14) (Coloured bars). Error bars show standard deviation (black lines). Mean scores were used for each participant post-biting and post-biopsy due to the range of scores depending on participant involvement and concordance with study procedures. Maximum possible score was 30. *p=0.02 using Wilcoxon matched-pairs signed rank test.

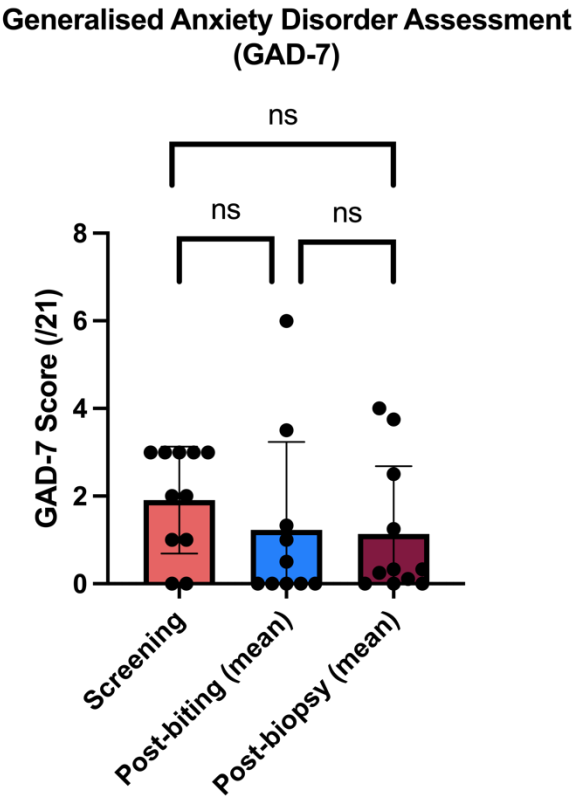

Figure 4.17: Scatter plot showing mean Generalised Anxiety Disorder Assessment scores.

At each time period, mean scores are given for all participants who underwent biopsy and had successful biting (n=11/14) (Coloured bars). Error bars show standard deviation (black lines). Mean scores were used for each participant post-biting and post-biopsy due to the range of scores depending on participant involvement and concordance with study procedures. Maximum possible score was 21. No significant difference noted between any pairing using Wilcoxon matched-pairs signed rank test.

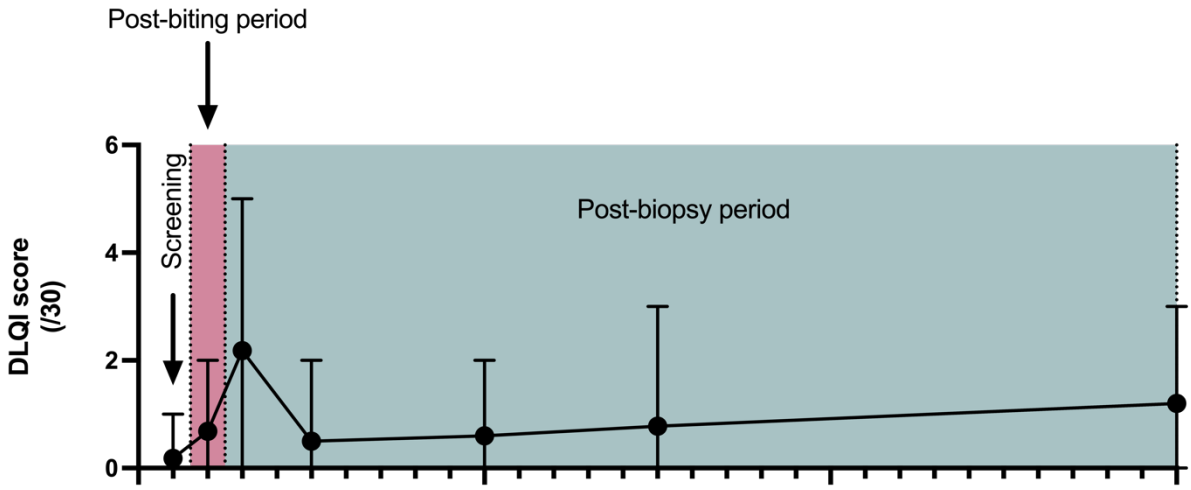

Figure 4.18: Graph to show trend in DLQI throughout study.

Mean scores with range are shown for each planned DLQI recording period (Screening, post-bite period (mean), Day 10, 30, 90, 180 post-biopsy).

Participants were asked to complete validated quality of life measures throughout the study, with the Dermatology Life Quality Index (DLQI) used to measure the impact of skin changes, and the Generalized Anxiety Disorder assessment 7 score (GAD-7) used to measure any mood disturbances. See Figure 4.16, Figure 4.17 and Figure 4.18 for comparison of scores during each phase of the study. Interpretation of the scores can be summarised as:

DLQI

- 0-1: no effect at all on participant's life
- 2-5: small effect on participant's life
- 6-10: moderate effect on participant's life
- 11-20: very large effect on participant's life
- 21-30: extremely large effect on participant's life

GAD-7

- 0–4: minimal anxiety
- 5–9: mild anxiety
- 10–14: moderate anxiety
- 15–21: severe anxiety

The minimal clinically important difference (MCID) has been defined as 'the smallest difference in score in the domain of interest which patients perceive as beneficial and which would mandate, in the absence of troublesome side effects and excessive cost, a change in the patient's management'.(Jaeschke, Singer and Guyatt, 1989). The mean change in score (comparing screening score to peak score) for all participants (n=14/14) for DLQI on a 30-point system, and for GAD-7 on a 21-point system (1.79±2.36 and 0.14±1.99 respectively; Mean±SD) were both below the suggested MCID (Toussaint et al., 2020; Basra et al., 2015).

The mean change in score for participants who sustained a successful bite (n=12/14) for DLQI and for GAD-7 on a 21-point system (1.92±2.54 and -0.17 ±1.94 respectively; Mean±SD) were also both below the MCID. No participants had a change in GAD-7 that was above the MCID. 3/14 participants (who had also all had successful sand fly biting) did have a change in DLQI scores that was above the MCID and therefore clinically significant. The

peak MCID score was 8/30 (moderate effect on participant's life), and this occurred following biopsy. The mean score for GAD-7 (1.18±1.47) and DLQI (0.87±1.38) for all participants was consistent with 'minimal anxiety' and 'no effect at all on patient's life' respectively.

4.3.4 Public and participant engagement videos

Credits:
Executive Producer: Vivak Parkash
Producer: Paul Reed (paulreedvideo.com)
Editing: Paul Reed (paulreedvideo.com), Vivak Parkash
Director of Photography: Dan Monro (Lookout Productions)

Figure 4.19: Stuart's story (video link):

https://www.youtube.com/watch?v=kkzRyLDskkI

Figure 4.20: Claire's story (video link):

https://www.youtube.com/watch?v=KiD0aJj4e7k

Figure 4.21: Ehren's story (video link):

https://www.youtube.com/watch?v=zF6sYyT3xzg

4.4 Discussion

Leishmania has long been considered a suitable organism for which a controlled human challenge model could be developed and could effectively test for vaccines (Mohebali, Nadim and Khamesipour, 2019b; Melby, 1991). I have described here a novel sand fly-initiated *Leishmania* CHIM, which demonstrates efficacy (attack rate of at least 64.29%, rising to 100% depending on method of calculation), and safety (no grade 3 or greater AEs, no SAEs or SUSARs). 3/14 (21.4%) of participants demonstrated evidence of parasite persistence and disease recurrence, which was responsive to further treatment. Figure 4.5:

Lesion development and parasitological confirmation summary, demonstrates both histological/immunohistochemical, clinical and qPCR data confirming parasite presence. See also Figure 7.35: LEISH_Challenge picture summary for all participants, for characterization of lesion development.

The numbers of participants who expressed interest and were pre-screened (n=105) demonstrated the interest such studies may attract in the post-COVID-19 era. 42.9% of participants were male at birth (n=6/14), and 57.1% (n=8/14) were female at birth, which was a more even distribution in comparison to the previous FLYBITE study. Representation is an important feature of clinical studies, as discussed in Chapter 3, and active efforts were made to target different ethnic groups during the recruitment phase, although investigators again noted a lack of representation. This is a feature common to many studies and may need an institutional and perhaps a governmental approach to change the research culture and attitudes surrounding this. Individuals with atopy were included in this study (see Table 4.6), although a clinical decision was made as to the degree of atopy. Individuals were typically excluded if there was a moderate to severe history, or a strong history of multiple atopic conditions, given the possible overlap with the propensity to anaphylaxis. Serum IgE was used as a screening tool for the degree of atopy and therefore anaphylaxis, although this is generally acknowledged to be poor for this use. Serum tryptase, a marker of mast cell degranulation, could be an adjunctive test, although has limitations such as logistical challenges in sample processing, and was not used here, although could be considered in future studies.

A number of metrics were used to aid in measuring effectiveness (sand fly bites, evidence of sand fly blood meal, clinically compatible lesion, qPCR and histological confirmation), all of which are relevant to potential for lesion development (see Figure 4.5 & Table 4.9). After 30 minutes of exposure to 5 *Leishmania major*-infected sand flies 78.6% (11/14) of participants demonstrated evidence of sand fly biting and 85.7% (12/14) of participants demonstrated evidence of clinical cutaneous leishmaniasis lesions. Given the 2/14 participants who demonstrated evidence of biting failure and subsequent failure of lesion development were actively replaced in the study as per the adaptive study design, this demonstrates a positive study outcome. Of the 14 participants, parasite confirmation (either or both of positive

qPCR result and histological confirmation of parasite presence in biopsy samples) showed firm confirmation in 64.3% (9/14) participants. In all but one case (LC027), the presence of visualised bites immediately after removal of the biting chamber correlated with a confirmation of parasite presence. LC027 had no evidence of sand fly biting, although did develop a clinically, but not parasitologically confirmed lesion. Of the 78.6% (11/14) of participants who had evidence of sand fly bites, 81.8% (9/11) of these participants had 1 or more sand flies from the sand fly biting chamber, demonstrate evidence of having taken a blood meal, with these 11 participants all developing clinically compatible lesions with parasitological confirmation. Presence of sand fly bites is a strong predictor therefore of CL development and suggest that infected sand fly feeding behaviour is highly efficacious at transmitting viable parasites to the skin of the host. The same sand fly batch was not used for each participant given the life cycle, and life expectancy of sand flies being less than 4 weeks. Across both cohorts, multiple batches were used, and so this behaviour does not suggest an effect from one particular batch of sand flies. There were more bites than sand flies that demonstrated evidence of blood in all successful biting episodes, except one (LC011). This is similar to the results seen in the FLYBITE study and confirms that although sand fly behaviour is affected by presence of *Leishmania* infection (Rogers and Bates, 2007; Beach, Kiilu and Leeuwenburg, 1985), the biting behaviour on humans remains consistent. CD4 count at screening may have some benefit in future studies, given those individuals with lower baseline CD4 counts developed recurrence after initial successful infection, and those with higher CD4 count had reduced lesion development (see Table 4.7). The numbers in each of these groups were small and there are a number of confounding variables, and so demonstrating significance is difficult and further assessment is needed.

The main objectives of this study were to demonstrate efficacious sand fly transmission of *L.major* which was also safe. The effectiveness of lesion development endpoints were primarily parasitogical confirmation determined by either characteristic immunohistochemistory and histopathology, qPCR, or following parasite culture from biopsy sections. Safety endpoints were determined by lack of study-associated SAEs or grade 3 AEs, and cutaneous leishmaniasis lesion clearance at 1 year follow -up. With respect to safety, if excision biopsy had not resulted in lesion clearance, then the endpoint included clearance following additional cryotherapy.

The primary lesion development endpoint was dichotomous, i.e. lesion presence vs no lesion presence. Following lesion development and subsequent biopsy there was quantifiable data to confirm parasite presence. However, the decision to proceed to biopsy and initially label a lesion as CL was clinical. The study protocol discusses this in detail, with a clinically compatible lesion defined as lesion is present at 14 days or more successful post-sand fly successful biting, which is papular, raised, erythematous and ≥3mm in diameter (see section 4.2). Some of the additional dermoscopy features of a CL lesion are discussed in chapter 1.

In order to generate robust evidence to inform further research, and subsequent implementation of practice, it is vital that clinical research has appropriate endpoints. Given the possibility of outcomes from studies leading to change in healthcare policy and informing vaccination research and strategy, this is particularly important with respect to vaccine-CHIM studies. An additional consideration is the possibility of licensure straight from a vaccine-CHIM if efficacy is confirmed, as was the case with the cholera vaccine (Chen et al., 2016). This could be relevant for disease processes where clinical endpoints in the field are difficult to achieve. To determine clinical effectiveness, precise endpoints reduce the possibility of bias and spurious results. The discussion of suitable and harmonized endpoints in CHIM studies has mainly focused on enteric organisms (Giersing et al., 2019; Porter et al., 2017, 2019).

The study design has attempted to control for confounders by a number of mechanisms including use of a well characterized strain of *L. major*, and standardized exposure to infected sand flies (preserved number of sand flies and exposure timeframe) following a robust screening process. Confounding issues such as host skin factors, variability in inoculum and number of sand fly bites (Kimblin et al., 2008) are challenging to control for and will have an effect on outcomes. Given the limited surrogate or biomarker-associated endpoints in CL, the outcome measures where relevant are clinical. Increasingly CHIM studies are however also focussing on immunologic endpoints (Porter et al., 2017).

There is an argument that the attack rate should closely mimic the natural infection process, given attack rates that are exaggerated compared to endemic settings may overwhelm the normal immune function. This could lead to a therapy not demonstrating protection in a controlled setting, but this may not represent the real-world manifestation. With respect to *Leishmania* infection, natural exposure is unlikely to involve 5 sand flies biting in a concentrated area. However, to avoid the possibility of widespread disease, and/or failure of the process this methodology was chosen, in conjunction with the PI exercise described in Chapter 2 (Parkash et al., 2021). Infectivity rates and colonisation of *Leishmania* parasites of the sand fly stomodeal valve is variable and is additionally dependent on species of both sand fly and *Leishmania* (Alexandre et al., 2020; Ashwin et al., 2021; Myskova et al., 2008). Therefore 5 sand flies were chosen as the optimal number.

Observed differences between this CHIM and the FLYBITE uninfected sand fly biting study suggest that presence of parasites results in lack of complete sand fly engorgement which is in keeping with the literature. In the cases where sand files had evidence of blood meal on dissection (71.4%, n=10/14), sand fly abdomens were only partially filled with blood as opposed to displaying full engorgement. Macroscopic visual inspection of sand flies was a poor predictor of presence of blood on dissection and subsequent infection, with only 30% (3/10) of these cases showing evidence of obvious macroscopic engorgement. In each of these 3 cases, only 1/5 sand flies were noted to have macroscopic evidence of engorgement. In contrast The mean number of sand flies that demonstrated evidence of blood meal, where blood was noted on dissection (for n=10/14 participants) was 2.5, falling to a mean of 1.8 bites across all (n=14/14) participants.

An interesting observation, as alluded to above and in the results section, is the relative heterogeneity of clinical findings in the presence of lesion development. All participants (100%, n=14/14) developed lesions close to or at the bite site, although only 12 were deemed clinically to be compatible with CL lesions. This distinction was based on rate of lesion development, similarity to other participants, appearance in comparison with the know progress of cutaneous leishmaniasis but also clinical and dermatological observations by myself, Professor Lacey and Professor Layton. The 2 participants were deemed to be biting failures (LC023 and LC012) were replaced in the study, although did develop small

non-clinically compatible lesions. LC023 had a lesion with a maximal diameter 2.28 x 2.62mm at Day 12 following biting. This participant had no compatible visible bites immediately following biting and no sand fly evidence of blood meal. This lesion continued to resolve from this point and had completely disappeared by Day 77 according to participant measurements. Given the small size, rapid resolution and limited correlation with a CL lesion, a discussion was had with the participant and the decision was made to monitor the lesion and avoid biopsy. LC012 similarly had no sand fly evidence of blood meal and no visible blood in the sand fly abdomen on dissection. This lesion did not develop until approximately day 35, reaching a size of 1mm x 1.5mm. This lesion was not clinically compatible with a CL lesion, however the decision was made to biopsy in conjunction with the participant in order to exclude the diagnosis of leishmaniasis, with biopsy being qPCR and histology negative for parasite presence. Both participants had no further sequelae out to 1-year post-resolution and post-biopsy respectively. It is possible that these non-clinically compatible lesions, are non-infected biting lesions, such as those noted in the FLYBITE study. These would either be a reaction that would be expected with any hematophagous insect bite, or a reaction to specific sand fly salivary gland proteins given their immunogenic potential (Abdeladhim, Kamhawi and Valenzuela, 2014; Taylor-Robinson, 2001; Kamhawi, 2000). The other possibility, given the lack of objective evidence of parasite presence is the possibility of self-resolution of a CL lesion. This could be explained by either an efficient immune clearance response in these individuals or a lower parasite load at time of inoculation. Other studies have noted that early healing lesion resolution within 3 months are possible (Oliveira-Ribeiro et al., 2017), although this could be considered less likely given the accepted time frame of self-heal is thought to be 6 months to a year. LC027 had no evidence of sand fly bites, and sand flies from their biting chamber had no evidence of blood meal and was therefore deemed a biting failure. At day 6 they developed 2 small erythematous blisters at the bite site. The blisters resolved but there was a residual macular lesion, 4.3mm x 4.7mm, by day 19, which was compatible with CL and fit the criteria for biopsy. Although qPCR and histology were only suggestive of inflammatory infiltrate rather than parasite presence, given the timeline and appearances, this was deemed a clinically compatible *Leishmania* lesion. This is suggestive of a highly efficient mechanism for infection, with sub-clinical sand fly biting. This may also have implication for disease control and spread of disease, given the possibility of subclinical exposure to infected sand fly bites.

The 2 cohorts each had a different range of time to biopsy (28-48 days and 14-26 days for cohort 1 and 2 respectively) (see Table 4.8). This was in part due to a protocol change prompted by rapid lesion development in cohort 1. The initial protocol dictated that biopsies should occur from day 28 post-biting onwards, to allow for distinguishing lesions from 'biting lesions', as observed in the uninfected biting study, and the observed phenomena of lesions usually taking months to develop (CDC website). Confounding factors included availability of participants to attend for biopsy, and timely reporting of lesion development via the electronic diary to allow for identification of clinically compatible lesions. It was observed however that lesions developed much earlier than those observed in the wild. This time to lesion development was similar to that noted in underpinning studies with the same isolate, MRC_02, in mice (Ashwin et al., 2021). It has also been noted that repeated blood meals and biting are associated with more rapid lesion development, which could account for the phenomena observed in this study (Serafim et al., 2018; Beach, Kiilu and Leeuwenburg, 1985; Vojtkova et al., 2021). The lesions that developed were rapid although had macroscopic and dermoscopic appearances consistent with cutaneous leishmaniasis. However, following biopsy, healing cohort 1 scars were noted to be inflammatory (see appendix Figure 7.35), remaining erythematous for several months. There are divergent opinions of persisting parasite following clinical cure of CL including in self-limiting lesion resolution (Sghaier et al., 2022; Martínez-Valencia et al., 2017), and so the persistent inflammatory lesions are difficult to attribute to residual parasite causing immune activation, versus a residual purely inflammatory process from the initial infection (Saldanha et al., 2020). 2 participants from cohort 1 developed recurrence of disease (LC001 & LC004). One of the participants (LC001) is thought to actually had a late presenting primary CL lesion rather than a recurrence. This recurrent lesion was within the region that the biting chamber aperture was placed, however it was not close to the primary lesion, and therefore was spared during biopsy. Upon its development, it resembled a suture mark, however after increasing in size it was identified as a CL lesion (see appendix Figure 7.35). As this cannot categorically be proven and given the late stage of the lesion development following biopsy it was most secure to label this as a recurrence. All cohort 1 participants who developed a clinically compatible lesion, successfully underwent a biopsy which was identified as containing *Leishmania* parasites, and there were 2 episodes of disease

recurrence. Cohort 2 participants who were identified as having a CL lesion, were not universally identified as having *Leishmania* parasites within biopsies, although had better cosmetic results compared to cohort 1 and lonely 1 episode of recurrence. Hence an earlier timepoint for biopsy may be associated with better participant outcomes, but poorer diagnostic yield.

The biopsy was the sole treatment to terminate the infection for primary infections, with the rationale being an anticipated significant reduction in parasites and pro-inflammatory factors, with a dependence on the host immune response to clear any residual parasites that were present in low number (Lestinova et al., 2017; Kaye and Scott, 2011). Given the subsequent observation of recurrence of disease, albeit in a minority of participants this could feasibly have occurred as a result of physical intervention, i.e. biopsy, causing a reactivation of leishmaniasis, as has been documented following trauma (Mendes et al., 2014; Mulvaney et al., 2009). This therefore leads to several conclusions, and potential considerations for future studies, namely a possibility of a watch and wait approach following lesion development to allow for self-heal; and potential use of anti-leishmanial chemotherapy such as topical miltefosine following any biopsy. There could also be a consideration for measuring the cytokine and chemokine response in PBMCs from participants to understand if a participant is in the healing or non-healing phase of lesion development (Moafi et al., 2017).

The heterogenous appearance of cutaneous leishmaniasis lesions in each cohort could be explained by the possible infectious dose from sand fly bites. There is relative heterogeneity in parasite load delivered from each sand fly bite, which subsequently has implications for disease development and lesion size (Giraud et al., 2019b; Kimblin et al., 2008). Within this study, given the limitation of repeat sampling on human subjects, it was not directly possible to measure the parasite load after each bite. In addition, the successful visible bites delivered, differed between individuals which may also impact on lesion development (Vojtkova et al., 2021). This could be compounded further even if each bite during a biting sensation does not deliver parasites, given the presence of other factors in sand fly saliva (both native sand fly and *Leishmania*-derived factors) which could both promote as well as protect against lesion development (Giraud et al., 2019a; Aoki et al., 2022; Lestinova et al.,

2017; Laurent et al., 2013; Oliveira et al., 2020; Taylor-Robinson, 2001; Teixeira et al., 2014; Vojtkova et al., 2021; Kamhawi, 2000; Abdeladhim, Kamhawi and Valenzuela, 2014). These factors are difficult to measure in this model and do not necessarily reflect natural infection, where multiple bites in one anatomical region are less likely.

There were some potential benefits to participation for participants outwith the reimbursement for time and altruism. These subtle benefits were difficult to quantify but may be important for future *Leishmania* CHIM studies, and also to other long-term clinical studies. At screening, participants underwent a thorough medical examination, and in some cases unexpected incidental medical findings led to further investigations and treatment, for which participants may not have otherwise sought medical treatment. Incidental findings are not uncommon but across both this study and the FLYBITE study, were relatively over-represented. Incidental findings are poorly described outside of studies involving cross-sectional imaging with limited studies reviewing incidental findings based on history and clinical examination (Hodgson et al., 2022). This reinforces the importance of thorough clinical examination and history taking. Furthermore, this also reinforces the importance of ensuring safety factors are at a minimum up to commonly accepted standards, given the possibility of including those individuals who may be unknowingly predisposed to reactions such as anaphylaxis. I propose that the clinical supervision, expertise and equipment that were used in this study, should be used in other clinical trials and studies that take place away from direct access to the hospital setting, such as those that take place in GP settings and of course in university settings. All participants also had an assessment of baseline psychological wellbeing. Any significant findings that were highlighted by this assessment were disclosed to the participants GP if consent for this has been given. This evaluation continued during the study and allowed for an extra layer of support and monitoring. Widespread use of psychological wellbeing are not yet universally incorporated into long-term studies. The method of data capture here is easy for participants to use and could be considered elsewhere. Exposure to sand fly resulting in a cutaneous leishmaniasis lesion, may for some individuals protect against future leishmaniasis infection after further exposure e.g. if participant travels to an endemic setting, given the cross-protection noted for with *L. major* and indeed uninfected sand fly bites (Tonui and Titus, 2007; Romano et al., 2015; Kamhawi et al., 2000b; Teixeira et al., 2014).

An adaptive clinical trial design was used in this study, and these have become more widely discussed recently, (Coffey and Kairalla, 2008; Drazen et al., 2016). Adaptive studies are now increasingly being regarded as useful and beneficial in specific circumstances within clinical research (Park, Thorlund and Mills, 2018). The last decade has seen an increasing use of adaptive designs, particularly in drug development and during the SARS-CoV-2 pandemic (Reis et al., 2021; Stallard et al., 2020). They frequently differ from conventional clinical trials as they allow modifications to key trial design components during the trial, as data is being collected, using pre-planned decision rules. The adaptive design used here allowed a checkpoint and subsequent change in protocol after the initial 6 participants, namely reduction in size of the biting aperture due to rapid lesion development, allowed for amendment of procedures surrounding participant replacement if noted to be 'biting failures', and allowed for earlier biopsy.

In this study, analysis of blood meal was undertaken by microscopy after dissection of sand flies following biting on participants. It is feasible that a small blood meal could have been missed in those cases were a blood meal was noted to be absent. Several methods have been described to detect blood meals in sand flies, and the use of these combined with microscopy would give firm evidence for bloodmeal in future biting studies on humans (Talebzadeh et al., 2023; Yared et al., 2019; Hlavackova et al., 2019; Yousefi et al., 2023; Garlapati et al., 2012).

Public and participant engagement in research studies is an important, and now burgeoning area of focus in research studies. I have shown here several interview videos taken with participants in these studies, both during the challenge itself, and shortly afterwards (see section 4.3.4)Public and participant engagement videos. These videos will be used to attract participants for any future studies, but also to attract a new audience to the subject matter. A planned focus group will take place in future to assess the acceptability of this study to participants, and also how they view these videos in terms of acceptability and accuracy, in an iterative process of participant input.

This study marked a major milestone in *Leishmania* focused vaccine research and also in the development of a novel vector-delivered CHIM. This CHIM model was efficacious and demonstrated safety and acceptability to participants. Future studies are likely to focus on maintaining participant approval, ensuring absence of recurrence, and demonstrating translation to testing of vaccine efficacy. The development of new candidate vaccines for leishmaniasis, (Osman et al., 2017),(Duthie et al., 2017),(Laurent et al., 2013)(Zabala-Peñafiel et al., 2020),(Zhang et al., 2020), provides a renewed focus to re-evaluate and update previous CHIM models.

5 Chapter 5: Concluding thoughts

5.1 Summary

Leishmaniasis affects hundreds of thousands of individuals every year, with a significant health burden demonstrated. There is a wide spectrum of disease, predominantly affecting tropical regions although new autochthonous cases are increasingly reported from temperate climates. The sand fly vector and the *Leishmania* parasite have co-evolved over thousands of years. The resulting infection process demonstrates complexity and exemplifies the importance of sand fly components in initiating infection. The spectrum of disease, from visceral leishmaniasis affecting internal organs to cutaneous leishmaniasis causing skin disease, is determined both by infecting species, but also host and vector factors. There have been multiple vaccines in clinical trials for human leishmaniasis, but not vaccine has yet reached market for clinical use. The re-emergence of deliberate human infection challenge in the form of controlled human infection models, has given renewed focus to develop a suitable model for leishmaniasis. Given the genetic homogeneity between *Leishmania* species and observed cross-protection in experiments involving differing species, a human infection model using *Leishmania major*, which causes exclusively skin disease, and which can be self-limiting, is thought to represent the best approach. The purpose of the work described within this thesis is the development of a novel CHIM for cutaneous leishmaniasis using sand fly-initiated infection on human participants. A small study reviewing cases of leishmaniasis imported to the UK, at a major infectious diseases centre, reiterated the need for enhanced surveillance to understand the scale of the problem. This also strengthens the need to ensure screening of potential volunteers for *Leishmania* human research when wishing to include *Leishmania*-naïve individuals in studies, even in non-endemic settings.

5.2 A novel CHIM for *Leishmania* would be acceptable to the public

Public engagement in various forms, is becoming more commonplace within research studies involving human subjects. Public engagement can include giving voices to both research participants and the public on their opinions on research conduct, novel findings, and future research direction. This can aid in making research more relevant, engendering a

culture of shared accountability, and improving awareness. Increasingly public involvement (PI) and patient-public involvement (PPI) group exercises are conducted at the outset of planned research activity to understand the public perceptions and ensure the research schedule aligns with public expectation and ethical practices. In the development of this CHIM, a PI activity was planned in the initial phase, with a series of planned focus groups at defined timepoints to determine the acceptability of the proceedings to participants (see Figure 1.6 for a description of the enabling studies on the pathway to a *Leishmania* CHIM).

The PI activity conducted at the outset determined much of the scope of the broader study. Participants included members of the public, a patient research ambassador, previous vaccine study participants and a CHIM study participant from a bacterial challenge study. The themes that resulted were similar to themes from other PI and PPI studies, and included the balance of altruism and financial reward as motivators for involvement in studies, the importance of clarity of the message given to potential participants, i.e. informed consent, and the suggested use of technology including social media to target new audiences and assist in conducting the research itself.

The PI group exercise also served to inform a new audience about *Leishmania* and other CHIM studies, both directly through informing of PI group volunteers, but also indirectly through publication of results and a strengthened outlook towards public involvement in the other aspects of the broader study. Known treatment options were also discussed and, unexpectedly, of these participants favoured an excision biopsy as a method for terminating the infection. This was both for the altruistic purpose of donating tissue that could yield a useful scientific purpose but also the mainly psychological connotation of 'removing' parasites and infection. This was not considered likely prior to the PI group and is therefore an amendment of the proposed methodology. Biopsy has been used in other skin challenge studies, but mainly as a method for sampling and confirming results, rather than influencing the course of infection. The theme of diversity also played a role in the PI group discussion. Study participants voiced concern about including both diverse groups that may have differing skin types relevant to infection, but also ensuring the results were applicable to endemic regions.

These findings indicate a sand fly initiated *Leishmania* CHIM would be an acceptable approach to test vaccines against cutaneous leishmaniasis.

5.3 A proposed protocol using sand flies demonstrates efficacious biting

The development of a sand fly biting protocol on human participants considered the previous PI activity and its impact on the proposed approach. Different approaches by other groups were considered when developing this protocol, on a background of a many other sand fly biting experiments on human participants over the past century.

Sand flies were used in a watch-like biting chamber device to facilitate exposure to participants. Of the two major vectors of *Leishmania major*, *P. papatasi* and *P. duboscqi*, it was not known which was more efficient at conferring infection and biting rates on humans. As such, 6 participants were recruited to a two- armed study using each sand fly species. Safety was monitored by both subjective and objective recording of localized and systemic effects, carried out at the time of biting and out to day 21 post biting. Visual analogue scores were used to help record this data. No grade 3 or higher adverse events were noted by investigators throughout the study, and the visual analogue scores remained low throughout the study. Maximum pain was reported as 2/10 for any one participant. There was no significant different between biting rates using either sand fly vector. On peripheral blood tests, inflammatory markers remained low throughout the study. Participants underwent a post-biting focus group following the final day 21 follow-up visit. The findings form the focus group suggested that uninfected sand fly biting was tolerable, especially when benchmarked against having blood draw for analysis, and the study procedures were overall well conducted from a participant point of view. Participants restated the need for adequate education of potential research participants given the complexity of CHIM studies and the implications of deliberate *Leishmania* infection.

5.4 A novel CHIM for *Leishmania* is effective and may be useful to test therapeutic agents

In addition to the above-mentioned underpinning activities in developing a novel CHIM for cutaneous leishmaniasis, in parallel our research group developed a novel parasite bank specifically designed for use in CHIM studies. In developing this bank, 2 new strains of *Leishmania major* from named patients were compared, and the patients were followed up to determine clinical outcomes. Transmission competence in sand flies, and infectivity in mice were demonstrated, as well as drug sensitivity testing using commonly used anti-leishmanial agents. Genomic sequence data confirmed the provenance of the strains and placement within the known phylogeny. A strain called MRC_02 was chosen from these based on the above favourable characteristics including rapid and predictable lesion development. A novel parasite bank to GMP was subsequently developed using this strain.

An adaptive design was then used in a formal *Leishmania major* CHIM study, to pragmatically expose the least number of participants to *Leishmania* to get a useful result (see Figure 4.3). Data from the parasite strain characterization study suggested a more rapid development and colonization in *P. duboscqi* compared to *P. papatasi* (Ashwin et al., 2021), as well as in other studies (Čiháková and Volf, 2016). These features combined with the greater robustness of *P. duboscqi* (Mukhopadhyay and Ghosh, 1999), favoured its use in this CHIM. If 5 or greater, of 6, participants did not develop compatible lesions, then for the remaining 6 participants *P. papatasi* would be used.

The primary objective was the development of an effective CHIM, which was safe for volunteers, with secondary objectives including determining rate of lesion development, immune response, as well as participant acceptability. Each participant was exposed to five *Phlebotomus dubosqi* sand flies infected with the *L. major* MRC_02 strain for 30 minutes. Lesions were terminated using punch and excision skin biopsy. 2 participants were replaced in the study due to either sand fly biting failure and subsequent failure of lesion development, as per protocol. Of those participants who developed clinically compatible lesions (12/14 participants (85.71%)); 9/12 participants (75%) had firm confirmation of parasite presence with qPCR +/- histological confirmation. (see appendix Figure 7.35: LEISH_Challenge picture summary for all participants). 3 participants developed recurrence of disease, which were treated effectively with cryotherapy. All participants remain free of further lesion development, although some scarring remains (see appendix Figure 7.35).

There were no serious adverse events reported. Analysis of tissue biopsies revealed novel spatial transcriptomic insights at an early stage of human CL disease (Parkash et al., 2024).

5.5 Problems arising during research

The COVID-19 pandemic came to prominence at the end of the FLYBITE study and prior to the ethical approval of the LEISH_Challenge study. This had two main effects on the research, 1) a change to research conduct to ensure safety of participants and study staff (see section 4.2.16), and 2) an altered research strategy involving more electronic communication methods and recording of results, such as use of remote study visits and electronic diary card and photo uploads. This was common to many clinical trials during the pandemic as well as a legacy of increased use of electronic methods in carrying out research (Boughey et al., 2021). Given the impact on many other studies, namely cessation of research activity, the continuation of the study was a feat (Hawila and Berg, 2021). Recruitment appeared unaffected, and perhaps bolstered by increasing cultural awareness of clinical trials, in contrast to experiences by some groups (Meskell et al., 2023).

Owing to various resource pressures as a result of the COVID-19 pandemic, staff support for the project was stretched. This included staff from the supporting hospital recalled to clinical and administrative duties due to service pressures. As a result, I created electronic work flows to allow for smooth follow-up of participants, including increased use of electronic data capture, and enhanced use of medical staff to maintain safety.

5.6 Future work and practical applications

The natural progression of this work would be the development of a vaccine-CHIM study. The more protracted discussion would focus on the suitability to head directly into a CHIM study, or whether confirmatory studies would be required. Given the complexities of developing CHIM studies, the UK remains an attractive place to conduct CHIMs given challenge agents are not deemed to be an Investigational Medical Product (IMP), thus avoiding additional layers of regulatory approval by organisations such as the Medicines and Healthcare products Regulatory Agency (MHRA). However, if an IMP is used in a vaccine-CHIM study, then the vaccine and the challenge agent would both be subject to formal

MHRA approval. Nonetheless the challenge agent used in this study was discussed with the MHRA and methods for release testing were agreed to ensure it was up to Good Manufacturing Practice (GMP) guidelines, and the study carried out to Good Clinical Practice (GCP). If a CHIM were to be conducted in an endemic setting, or other setting outside the UK, then the challenge agent may be subject to additional regulatory approval. A CHIM in an endemic region may better profile the target stakeholders for future vaccination strategy. Although such a CHIM would face the challenge of demonstrating vaccine immunity in previously *Leishmania* or sand fly exposed individuals. Indeed, where patients have only been exposed to sand fly biting, this could affect perceived vaccine induced protection in many ways (Kamhawi et al., 2000a).

Within the context of these frameworks, one consideration could be to ask the LEISH_Challenge study participants if they wished to either be re-challenged with the *Leishmania* to test the likely hypothesis that immunity from infection would be durable, or to take part in early phase vaccine studies. A formal vaccine-CHIM would however require MHRA approval, although this could be advantageous in gaining future licensure. Although CHIM studies are not usually a substitute for efficacy trials, some exceptions exist however, with the cholera vaccine, Vaxchora, gaining licensure based on data from a vaccine-CHIM trial (Chen et al., 2016; Ramanathan et al., 2019).

Studies using an ambulant or outpatient delivered CHIM are now commonplace (Waddington et al., 2014), most recently enhanced by better technology allowing for video communication and electronic data capture. With this in mind, greater use of technologies may lead to more virtual follow-up visits. The experience of use of dermatoscopes within this study suggests that they can be used more widely given ease of use, relatively cost effectiveness, and ability to customise to different personal smart phones. Future studies may therefore make use of personal dermatoscopes to allow for detailed remote picture uploads using smart phones. Use of technology could also facilitate a 'hub and spoke' model for conducting a clinical trial, given the larger numbers of participants that may be required and the limited pool of potential participants in the local area. This approach has been used for other clinical trials with success (Adams et al., 2019).

Use of controlled human infection for cutaneous leishmaniasis could have some disadvantages and limitations. Although cutaneous leishmaniasis infection accounts for the largest disease and morbidity burden, visceral disease accounts for the majority of leishmaniasis associated deaths. Although some species have the potential to cause both types of leishmaniasis, *Leishmania major* used in this study is known to cause only cutaneous disease. Cross-protection across species has however been reported(Tonui and Titus, 2007; Romano et al., 2015; Duarte et al., 2016), but is not necessarily the natural progression of infection. As such the application of novel strains to this CHIM model, may provide a pathway to use of species that demonstrate visceral disease, although they may revert to wildtype and cause unexpected disease outcomes (Soto et al., 2021; Duarte et al., 2016; Uzonna et al., 2004; Fiuza et al., 2016; JC et al., 2017; Karmakar et al., 2021). In particular a novel *L. infantum* strain exhibited heterologous protection in both Old World and New World species (JC et al., 2017) and an *L. major* centrin knockout showed cross protection with *L. donovani* infection (Karmakar et al., 2021) – both in pre-clinical studies. The challenge strain was free of Leishmaniaviruses (LRVs), which although poorly understood do have an impact both positively and negatively on disease progression (Saura et al., 2022; Adaui et al., 2016; Abtahi et al., 2020), in some respects mimicking the Wolbachia paradigm (Bi and Wang, 2020). Future studies could make use of *L.major*-infected LRV if which treatment failure is noted despite efficacy in a vaccine CHIM, to better mimic wildtype strain infection.

6 References

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7 Appendix

LEISH CHALLENGE:

PATIENT AND PUBLIC INVOLVEMENT GROUP

Tuesday 12th February 2019, 10.00am – 12.00pm

Room RCH/042 (Pod 1), Ron Cooke Hub, Heslington East Campus

Agenda

Outline of the meeting		Professor Georgina Jones
Welcome and introductions		Professor Georgina Jones
Background to the study		Dr Alison Layton Professor Charles Lacey Dr Vivak Parkash
<u>Facilitated sessions</u>		Professor Georgina Jones
1.	Potential ethical issues relating to the studies	(*enclosures 1 and 2*)
2.	Recruitment and feasibility of meeting inclusion criteria	(*enclosures 1 and 2*)
3.	Plain English Summary of the research project	(*enclosure 3*)
4.	Review of advert	(*enclosure 4*)
Summary of meeting		Professor Georgina Jones

PPI/February 2019

Figure 7.1: Public Involvement group agenda

LEISH CHALLENGE:
PATIENT AND PUBLIC INVOLVEMENT GROUP

Participant Information Sheet – General Data Protection Regulation (GDPR)

Background

The University of York would like to invite you to take part in the following research project, "Development of a human challenge model of *Leishmania Major* infection as a tool for assessing vaccines against leishmaniasis".

Before agreeing to take part, please read this information sheet carefully and let us know if anything is unclear or you would like further information.

What is the purpose of the study?

The leishmaniases are global diseases impacting millions. No vaccine against human leishmaniasis has yet been developed. What is needed is a rapid, cost effective means for early evaluation of the efficacy of candidate vaccines.

The solution being adopted for other diseases, which we now wish to adopt for leishmaniasis is to develop a human challenge model as an integral part of the vaccine development pipeline.

Controlled human infection model (CHIM) studies involve deliberate exposure of human volunteers to infectious agents. CHIM studies have been conducted over hundreds of years and have contributed vital scientific knowledge that has led to advances in the development of drugs and vaccines. Current diseases for which human challenge models are available include malaria, dengue fever, pneumonia, cholera, influenza and RSV infection.

Why have I been invited to take part?

You will help us by assessing acceptability towards two clinical study protocols, to secure views on the preferred approaches to conducting them. You do not need to have any previous experience of research, and no formal qualifications are needed.

As a member of the group, you will specifically: consider the draft study protocols to identify any issues; ensure that the designs of the clinical studies are optimal; consider the ethical issues relating to the study; consider the plain English summary of the project; consider recruitment to the studies, and the feasibility of the inclusion criteria.

Do I have to take part?

No, participation is optional. If you do decide to take part, you will be given a copy of this information sheet for your records and will be asked to complete a participant consent form. If you change your mind at any point during the study, you will be able to withdraw your participation without having to provide a reason.

On what basis will you process my data?

Under the General Data Protection Regulation (GDPR), the University has to identify a legal basis for processing personal data and, where appropriate, an additional condition for processing special category data.

In line with our charter which states that we advance learning and knowledge by teaching and research, the University processes personal data for research purposes under Article 6 (1) (e) of the GDPR:

Processing is necessary for the performance of a task carried out in the public interest

Special category data is processed under Article 9 (2) (j):

Processing is necessary for archiving purposes in the public interest, or scientific and historical research purposes or statistical purposes

Research will only be undertaken where ethical approval has been obtained, where there is a clear public interest and where appropriate safeguards have been put in place to protect data.

In line with ethical expectations and in order to comply with common law duty of confidentiality, we will seek your consent to participate where appropriate. This consent will not, however, be our legal basis for processing your data under the GDPR.

How will you use my data?

Data will be processed for the purposes outlined in this notice.

Will you share my data with 3rd parties?

No. Data will be accessible to the project team at York only.

Anonymised data may be reused by the research team or other third parties for secondary research purposes.

How will you keep my data secure?

The University will put in place appropriate technical and organisational measures to protect your personal data and/or special category data. For the purposes of this project we will store data on dedicated servers and / or on the University of York central data store, which provided secure long term storage for data, including daily backups, according to the University of York Research Data Management Policy.

A study master file will be created to include the study protocol, original signed consent forms and ethics/governance documentation. It will also include a list of PPI group participants, their email address and their telephone number. The study master file will be stored at the York Biomedical Research Institute in a secure, fire and rodent-proof cabinet that is only accessible to authorised members of staff.

Information will be treated confidentiality and shared on a need-to-know basis only. The University is committed to the principle of data protection by design and default and will collect the minimum amount of data necessary for the project. In addition, we will anonymise or pseudonymise data wherever possible.

Will you transfer my data internationally?

No. Data will be held within the European Economic Area in full compliance with data protection legislation.

Will I be identified in any research outputs?

No.

How long will you keep my data?

Data will be retained in line with legal requirements or where there is a business need. Retention timeframes will be determined in line with the University's Records Retention Schedule.

What rights do I have in relation to my data?

Under the GDPR, you have a general right of access to your data, a right to rectification, erasure, restriction, objection or portability. You also have a right to withdrawal. Please note, not all rights apply where data is processed purely for research purposes. For further information see, https://www.york.ac.uk/records-management/generaldataprotectionregulation/individualsrights/.

Questions or concerns

If you have any questions about this participant information sheet or concerns about how your data is being processed, please contact xx in the first instance. If you are still dissatisfied, please contact the University's Acting Data Protection Officer at dataprotection@york.ac.uk.

Right to complain

If you are unhappy with the way in which the University has handled your personal data, you have a right to complain to the Information Commissioner's Office. For information on reporting a concern to the Information Commissioner's Office, see www.ico.org.uk/concerns.

Figure 7.2: Public involvement group participant information leaflet

LEISH CHALLENGE:

PATIENT AND PUBLIC INVOLVEMENT GROUP

Remit and Role Description

What is public involvement in research?

Public involvement is where people are not the subjects of research but are working with researchers to plan, manage and carry out research.

This includes offering advice as members of a public involvement group, and commenting and developing research materials. The public can help bring unique, invaluable insights and may have personal knowledge and experience of the research topic.

What is the project about?

The leishmaniasis represent a diverse collection of diseases. They affect approximately 150 million people in 98 countries and are recognised by WHO as major neglected diseases of poverty, and disproportionately affect populations in low and middle income countries (LMICs).

Visceral leishmaniasis results in more than 20,000 deaths a year; cutaneous leishmaniasis impacts quality of life for millions of people.

Treatment for cutaneous leishmaniasis has changed little in the last 50 years. Availability of an effective vaccine would have a major impact on health and economic development in LMICs where leishmaniasis is endemic.

Progression of leishmaniasis vaccine candidates is hindered by the absence of a ready way to evaluate efficacy, given that low natural transmission rates of leishmaniasis make the clinical path challenging, time-consuming and costly.

The solution being adopted for other diseases, which we now wish to adopt for leishmaniasis is to develop a human challenge model as an integral part of the vaccine development pipeline. Controlled human infection model (CHIM) studies involve deliberate exposure of human volunteers to infectious agents. CHIM studies have been conducted over hundreds of years and have contributed vital scientific knowledge that has led to advances in the development of drugs and vaccines. Current diseases for which human challenge models are available include malaria, dengue fever, pneumonia, cholera, influenza and RSV infection.

What would my role involve as a member?

You will help us by assessing acceptability towards two clinical study protocols, to secure views on the preferred approaches to conducting them.

You do not need to have an previous experience of research, and no formal qualifications are needed.

As a member of the group, you will specifically:

- Consider the draft study protocols to identify any issues.
- Ensure that the designs of the clinical studies are optimal.
- Consider the ethical issues relating to the study.
- Consider the plain English summary of the project.
- Consider recruitment to the studies, and the feasibility of the inclusion criteria.

What will the meeting involve?

- There will be just one PPI group meeting of approximately 10 members, to be held at the University of York, Heslington East Campus.
- The meeting should take no more than 2 hours, and will be held on **12th February 2019**, 10.00am – 12.00pm. Refreshments will be provided.
- An independent facilitator will run the meeting.
- The clinical team members on the project will attend to present to the group and answer any direct questions about the proposed clinical studies.
- The Project Manager will attend the meeting to take detailed notes, and with group members' permission, record the discussions and prepare a transcript.
- The Project Manager and Clinical Team will not participate in the discussions.
- An Agenda for the meeting will be provided to all group members, but the aim is to encourage open conversation and discussion.
- Following the meeting, a detailed summary will be circulated to all group members to ensure there is a correct reflection of discussions. The summary will by anonymised. These will be used to inform the design and delivery of the two clinical studies.

What will I receive?

Payment for your time will be provided in the form of a £75 Amazon voucher. Your travel expenses to and from the meeting will be reimbursed.

How do I claim expenses?

The Project Manager will provide a travel expenses form and further information on how to claim.

Please note, original receipts must support all expense claims.

Who do I contact for further information?

Full details of the project can be seen at: **www.leishchallenge.org**

If you would like further information or have any queries, please contact:

Liz Greensted, Project Manager
Email: liz.greensted@york.ac.uk, Tel: 01904 328621

This project: "*Development of a human challenge model of Leishmania major infection as a tool for assessing vaccines against leishmaniasis*" is funded by the Medical Research Council and Department for International Development (ref: MR/R014973/1)

MRC Medical Research Council

Department for International Development

Figure 7.3: Public involvement group role description

LEISH CHALLENGE:

PATIENT AND PUBLIC INVOLVEMENT GROUP

Consent Form

Thank you for agreeing to take part in our Patient and Public Involvement Group.

By signing this form you are agreeing to take part but this does not stop you from changing your mind at any point.

1. I confirm I have read and understand the PPI Group Remit and Role Description.
2. I agree to the PPI Group meeting being audio recorded.
3. I agree to the use of **anonymised** quotes in any publications that might ensue.
4. I agree to providing contact details and confirm I have read the Information Sheet for General Data Protection Regulations (GDPR).
5. I understand that, for my participation, I will receive a one off payment of £75, plus travel expenses, to be claimed after the meeting under the rules of the University of York Financial Regulations.
6. I would like to be kept informed of any results from the focus group - Y / N

Name:

Email address:

Telephone number:

Signature: ……

Date:

Figure 7.4: Public involvement group participant consent form

Plain English summary of the research programme

The following summary explains what we are planning on doing in our research project.

Our research relates to an infection called leishmaniasis. The infection is caused by a parasite called *Leishmania* that is transmitted by flies called sand flies. It mainly occurs in hot and tropical countries, affects millions of people and causes around 20,000 deaths across the world every year. There are different types of leishmaniasis around the world and some can be very serious. They affect the skin (cutaneous leishmaniasis) or the internal organs of the body (visceral leishmaniasis). Some of the milder forms will produce skin problems which will be localised, whilst other forms of leishmaniasis will cause widespread skin changes. We do have some treatments for leishmaniasis but many of them are not easy to use or don't work well. Therefore, we need new treatments and would like to find vaccines that prevent or work against leishmaniasis.

With funding which has been provided from the Medical Research Council we want to develop new treatments and vaccines against leishmaniasis. We want to work out how to give a volunteer a standard infection in the skin using a mild strain of *Leishmania,* and then treat it early and effectively. We could then use this system to test new vaccines and drugs to prevent or cure the skin infection. This is called a 'human challenge model'. There are a number of stages to this.

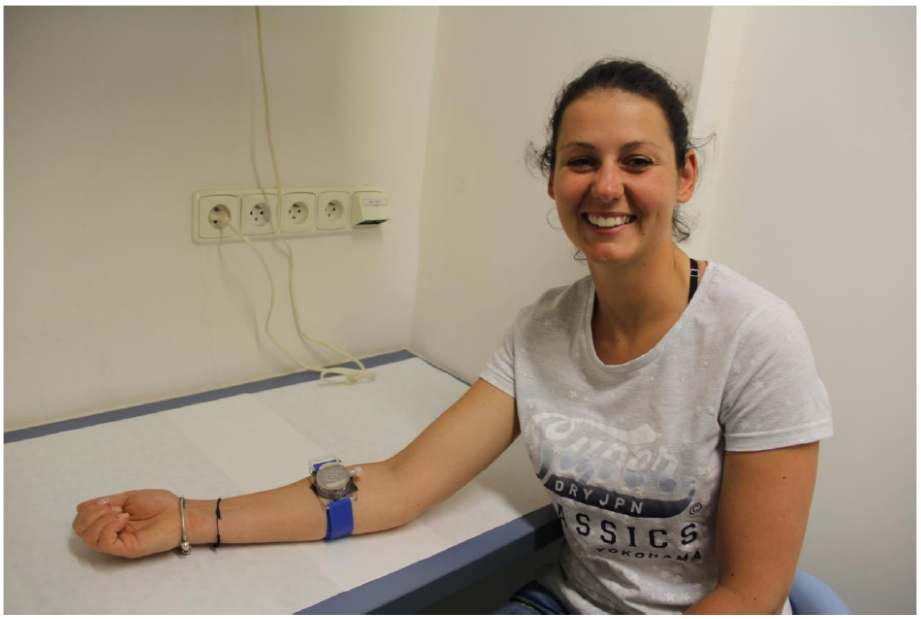
First of all, we are working with doctors in Israel who are part of our research group. In Israel people can get one of the mild forms of cutaneous leishmaniasis. We are taking some Israeli patients with leishmaniasis caused by a mild strain and are growing a pure form of the infection in the laboratory. We will do lots of tests to make sure we have grown pure infection.

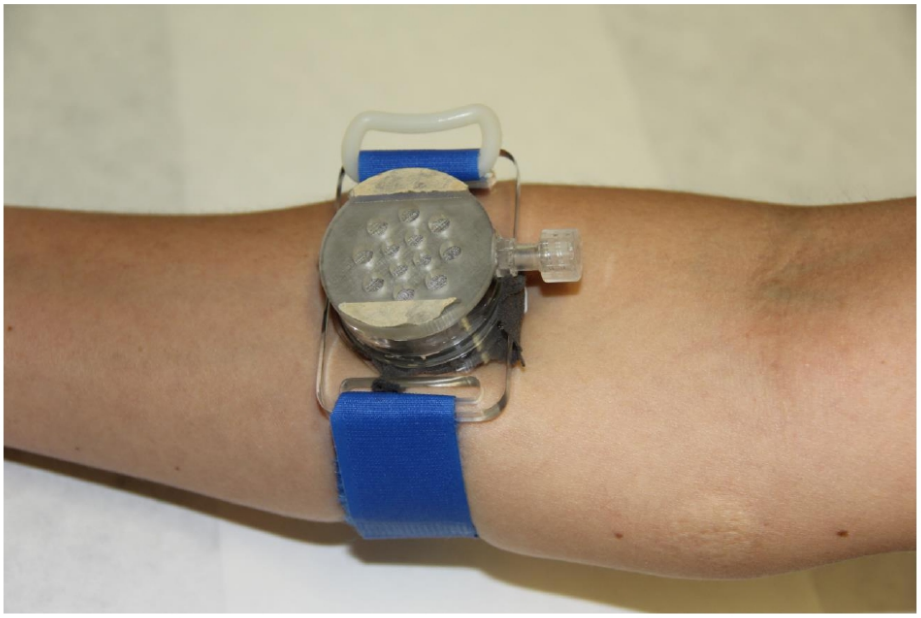
Secondly we are working with scientists in the Czech Republic who are 'insect experts' (entomologists). They are going to check that the Leishmania parasite we grow from the Israeli patients can infect sand flies and behaves the same way.

Then, third and fourth, we are going to carry out two studies here in York that we want your opinions about.

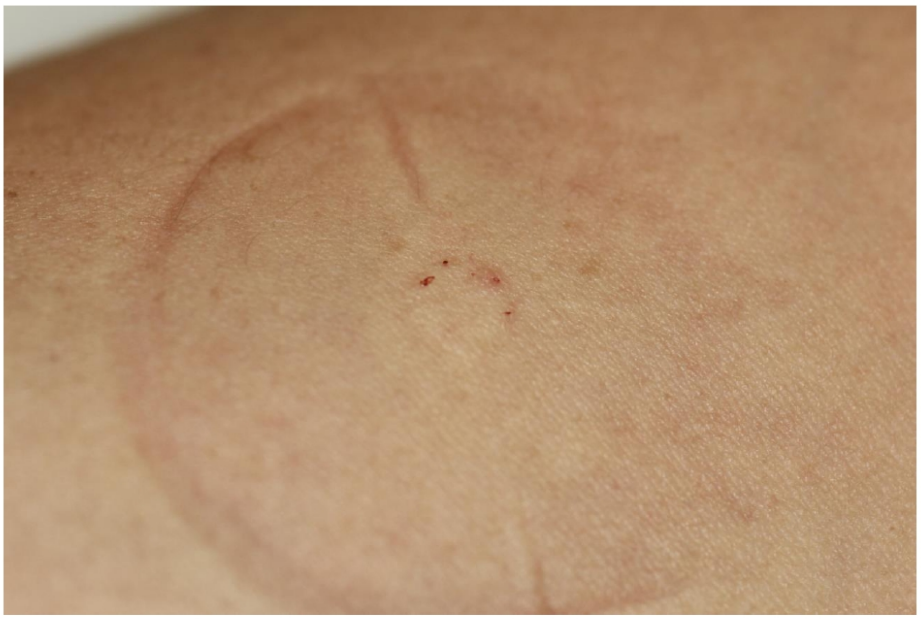
In the first study we want to set up a way for sand flies to bite volunteers in a controlled manner. This has already been done in the USA so we have a reasonable idea how to do this. In this study we will have tested the sand flies so they don't have any infection, and we will call this the **"uninfected biting study"**. The sand flies are maintained by our colleagues in the Czech Republic who will have carefully checked that they don't carry any infections. They will be kept safely at the University of York once they have arrived from the Czech Republic.

For the **"uninfected biting study"** we will advertise for 12 volunteers in the University and local area. Our study doctor would check them to make sure they are healthy and we would do a blood test to make sure they aren't allergic to sand flies. They would come in to our clinic at the University, and we would attach a gadget with the sand flies in it to their arm. Here are some pictures of what it would look like -





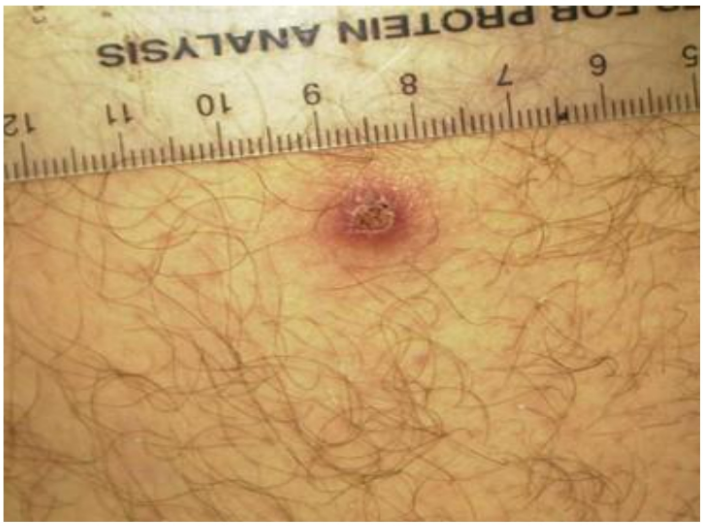
And here is what it looks like after the sand flies have bitten a subject:



Further details of the **"uninfected biting study"** are provided in the patient information leaflet, which we would also like you to consider.

The second study, which we will call the **"infected biting study"**, will take place at University of York. Sand flies would be fed on blood which contains the mild strain of *Leishmania* infection. Then we would repeat the biting study with infected sand flies. The gadget would be left for 10 - 20 minutes until the sand flies have bitten, and then it would be taken off.

We expect that over 4 to 12 weeks an early and localised skin lesion with the *Leishmania* infection will develop. We will see the volunteers regularly every week for the first 2 weeks then every 2 weeks to check their bites. We will use an instrument called a dermatoscope to check and magnify the area where the bites have occurred and will photograph the affected area through the dermatoscope. We anticipate that a lump or spot will develop and slowly expand. It may feel a little sore and itchy and should look like this:-



We will monitor the lump with the research subject, and once it is established as a cutaneous leishmaniasis lesion we will start treatment. This is likely to be when it is still quite small. The doctors that treat leishmaniasis in Israel tell us that if you start treatment when the spot is small it goes away and after treatment you can hardly see where it originally was. There are a number of treatments we can use and will choose the most appropriate one with the volunteer. These include an ointment that the volunteer can put onto the bump/spot, removing the lump with a small surgical procedure, freezing, or a one off treatment with a heat machine after using a local anaesthetic cream. Some of the treatments can cause some temporary discomfort and may result in a small scar. We will discuss with each person what kind of treatment they would prefer and some of these treatments could be combined.

Further details of the **"infected biting study"** are provided in the patient information leaflet, which we would also like you to consider. We would like your feedback on the overall plan, and there are various specific questions that we will ask you as well. Thank you for agreeing to advise us on our research.

Figure 7.5: Public involvement group plain English summary

Enclosure 4

Volunteers wanted
for clinical research study

York Biomedical Research Institute at the University of York are looking for healthy volunteers aged 18-65 to be part of a sand fly biting study.

Volunteers will be reimbursed for their time and expenses.

For further information.
E: liz.greensted@york.ac.uk
E: vivak.parkash@york.ac.uk
T: 01904 328621

LEISH Challenge

HULL YORK MEDICAL SCHOOL

UNIVERSITY of York

Figure 7.6: Public involvement group advertisement

LEISH CHALLENGE:
UNINFECTED SAND FLY BITING STUDY

Participant Information Sheet

Background

The University of York would like to invite you to take part in a research project, "Development of a human challenge model of *Leishmania major* infection as a tool for assessing vaccines against leishmaniasis". This is an initial study to test safety and review the study protocol using **uninfected** sand flies.

Before agreeing to take part in this research, please read this information sheet carefully and let us know if anything is unclear or you would like further information.

What is the purpose of the study?

Leishmania is a small parasite that infects human beings and can cause various diseases which we call leishmaniases. The parasites are transmitted by small biting flies called sand flies, mainly in tropical and warmer countries. There are millions of people worldwide with the various types of leishmaniases. No vaccine against human leishmaniasis has yet been shown to be effective. A system for early testing of candidate vaccines would be extremely useful in vaccine development.

A solution being adopted for other diseases, which we now wish to adopt for leishmaniasis is to develop a human challenge model as an integral part of the vaccine development pipeline.

Controlled human infection model (CHIM) studies involve deliberate exposure of human volunteers to infectious agents. CHIM studies have been refined in the last 20 years. They have contributed vital scientific knowledge that has led to advances in the development of drugs and vaccines. Current diseases for which human challenge models are available include malaria, dengue fever, pneumonia, cholera, influenza and RSV infection.

The first step in developing this CHIM is to expose human volunteers to **uninfected** sand flies, in order to assess the protocol for sand fly biting and review the safety aspects.

This is necessary before proceeding to the last stage of CHIM development; using sand flies infected with a form of the disease known as cutaneous leishmaniasis which will form a separate study.

Why have I been asked to take part?

Volunteers must be men and women aged between 18 and 65 years old. Enrolled female participants must **not** be pregnant or breastfeeding, and must be using a reliable form of contraception.

Volunteers must be healthy and **not** be at risk of serious infections and must **not** have any chronic skin conditions. Volunteers should **not** have travelled in the last 30 days to any area where leishmanasis is present. There should be **no** history of significant reaction to insect bites.

What does the uninfected sand fly biting study involve?

This study is designed to test safety and assess the sand fly biting protocol in healthy volunteers.

- There will be 4 visits in total, each lasting between 30 minutes to 2 hours. These will take place in York (time, date and venue to be confirmed).
- Prior to the study, blood tests will be performed to assess suitability. This is likely to take place a few weeks before the main study.
- The 2nd visit will be the biting visit itself. Using a custom wrist watch-like feeding chamber, up to 4 uninfected sand flies will be placed against the volunteers' skin.
- The exposure will be for 30 minutes.
- Blood tests will be performed following the study to help assess safety aspects of the study.
- Photographs will be taken of the bite site.
- The volunteers will be observed for a period of time before being allowed to go home. A doctor, nurse and an insect specialist (entomologist) and other senior study investigators will be present throughout.
- For any local skin reactions, antihistamine medications and creams will be offered.
- Follow-up visits will take place approximately at 3 days and 10 days after the biting study.
- Following the study, non-identifiable information will be recorded and will contribute to future publication and research.

Who is organising and funding this study?

Medical Research Council and Department for International Development (Ref: MR/R014973/1)

What will happen if I agree to take part?

You will be remunerated for your time and for travel expenditure. You will be contacted by the study team who will discuss the study in further detail including asking further questions to confirm eligibility.

What are the potential risks of taking part?

Volunteers may experience a 'pin-prick' sensation when bitten by a sand fly. Common effects following sand fly bites (as with all insect bites) include some swelling, pain and redness at the bite site. This may take up to 48 hours to develop. Just as with any insect bite there is the potential for a skin infection which may require antibiotic treatment.

To-date, there have been no reported cases of anaphylactic (life-threatening) reactions to sand fly bites in the whole medical literature. However, there might still be a small risk of serious reaction to sand fly bite which may include breathing problems, widespread skin rash and very rarely death. Blood tests taken prior to the study will help to identify volunteers who are more likely to suffer adverse effects and these individuals will be excluded from participating further.

Volunteers will undergo a blood test a few weeks before and shortly after the sand fly bite study. There is a small risk of bruising at the site from where the blood sample is taken. There may be a need for volunteers to return for additional testing at a later date.

If you feel unwell at any point during or after the study you will be asked to stop and be supported in receiving any necessary medical attention.

What are the potential benefits of taking part?

The information from this study will help to further understanding of sand fly bites and contribute to developing a model for testing vaccines using infected sand fly bites. Ultimately this research will help in the effort to eradicate leishmaniasis.

Do I have to take part in the uninfected biting study?

No, participation is optional. If you do decide to take part, you will be given a copy of this information sheet for your records and will be asked to complete a participant consent form. If you change your mind at any point during the study, you will be able to withdraw your participation without having to provide a reason.

What will happen to my samples?

The blood samples will be tested in the Centre for Immunology and Infection, Department of Biology, University of York and York Hospitals NHS Foundation Trust. This is where they will be studied by the research team. Samples will be tested in secure laboratory facilities accessible only by authorised research staff. Samples will be labelled with a unique ID number and the date only but no personal details, so they cannot be identifiable as having been donated by you.

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What happens if there is a problem?

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A study master file will be created to include the study protocol, original signed consent forms and ethics/governance documentation. It will also include a list of study group participants, their email address and their telephone number. The study master file will be stored at the Centre for Immunology and Infection in a secure, fire and rodent-proof cabinet that is only accessible to authorised members of staff.

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Will you transfer my data internationally?

No. Data will be held within the European Economic Area in full compliance with data protection legislation.

Will I be identified in any research outputs?

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This project: "*Development of a human challenge model of Leishmania major infection as a tool for assessing vaccines against leishmaniasis*" is funded by the Medical Research Council and Department for International Development (ref: MR/R014973/1)

MRC Medical Research Council

Department for International Development

Figure 7.7: Proposed participant information leaflet for future uninfected sand fly biting study – for use by participant involvement group only

LEISH CHALLENGE:
INFECTED SAND FLY BITING STUDY

Participant Information Sheet

Background

The University of York would like to invite you to take part in a research project, "Development of a human challenge model of *Leishmania major* infection as a tool for assessing vaccines against leishmaniasis".

Before agreeing to take part in this research, please read this information sheet carefully and let us know if anything is unclear or you would like further information.

What is the purpose of the study?

Leishmania is a small parasite that infects human beings and can cause various diseases which we call leishmaniases. The parasites are transmitted by small biting flies called sand flies, mainly in tropical and warmer countries. There are millions of people worldwide with the various types of leishmaniases. No vaccine against human leishmaniasis has yet been shown to be effective. A system for early testing of candidate vaccines would be extremely useful in vaccine development.

A solution being adopted for other diseases, which we now wish to adopt for leishmaniasis is to develop a human challenge model as an integral part of the vaccine development pipeline.

Controlled human infection model (CHIM) studies involve deliberate exposure of human volunteers to infectious agents. CHIM studies have been refined in the last 20 years. They have contributed vital scientific knowledge that has led to advances in the development of drugs and vaccines. Current diseases for which human challenge models are available include malaria, dengue fever, pneumonia, cholera, influenza and RSV infection.

This study is the last stage of CHIM development; using sand flies infected with a form of the disease known as cutaneous leishmaniasis. The study will use sand flies to bite volunteers and look at the development of a small lesion on the skin, which will then be treated with effective treatments.

Why have I been asked to take part?

Volunteers must be men and women aged between 18 and 65 years old. Enrolled female participants must **not** be pregnant or breastfeeding, and must be using a reliable form of contraception.

Volunteers must be healthy and **not** be at risk of serious infections and must **not** have any chronic skin conditions. Volunteers should **not** have travelled in the last 30 days to any area where leishmanasis is present. There should be **no** history of significant reaction to insect bites.

What does the infected sand fly biting study involve?

This study will give healthy volunteers a skin lesion (cutaneous leishmaniasis) and will use treatment at an early stage to resolve the infection.

- There will be up to 9 visits in total, each lasting between 30 minutes to 2 hours. These will take place in York (time, date and venue to be confirmed).
- Prior to the study, blood tests will be performed to assess suitability. This is likely to take place a few weeks before the main study.
- The 2nd visit will be the biting visit itself. Using a custom wrist watch-like feeding chamber, up to 4 infected sand flies will be placed against the volunteers' skin.
- The exposure will be for 30 minutes.
- Blood tests will be performed following the study to help assess safety aspects of the study.
- Photographs will be taken of the bite site.
- The volunteers will be observed for a period of time before being allowed to go home. A doctor, nurse and an insect specialist (entomologist) and other senior study investigators will be present throughout.
- For any local skin reactions, antihistamine medications and creams will be offered.
- Volunteers will then be seen at regular intervals in order to follow-up the lesion at the bite site. Volunteers will be monitored closely until the lesion is 3 - 5mm in size.
- Treatment will then be given to treat the lesion. There are a range of current effective treatments.
- Any patients who do not develop a lesion at the bite site will be followed up until 6 months following the study.
- Following the study, non-identifiable information will be recorded and will contribute to future publication and research.

Who is organising and funding this study?

Medical Research Council and Department for International Development (Ref: MR/R014973/1)

What will happen if I agree to take part?

You will be remunerated for your time and for travel expenditure. You will be contacted by the study team who will discuss the study in further detail including asking further questions to confirm eligibility.

What are the potential risks of taking part?

Volunteers may experience a 'pin-prick' sensation when bitten by a sand fly. Common effects following sand fly bites (as with all insect bites) include some swelling, pain and redness at the bite site. This may take up to 48 hours to develop. Just as with any insect bite there is the potential for a skin infection which may require antibiotic treatment.

To-date, there have been no reported cases of anaphylactic (life-threatening) reactions to sand fly bites in the whole medical literature. However, there might still be a small risk of serious reaction to sand fly bite which may include breathing problems, widespread skin rash and very rarely death. Blood tests taken prior to the study will help to identify volunteers who are more likely to suffer adverse effects and these individuals will be excluded from participating further.

The aim of this study is to cause a single lesion (spot) of cutaneous leishmaniasis to develop. Volunteers will therefore develop a lesion that will grow over a few weeks at the site of the bite. Treatment will be given to treat this lesion after it is allowed to grow to 3-5mm.

There is a small potential for scarring at the lesion site, however treatment will be given at an early stage to help minimise this risk.

Volunteers will undergo repeat blood tests a few weeks before and shortly after the sand fly bite study. There is a small risk of bruising at the site from where the blood sample is taken.

There may be a need for volunteers to return for additional testing at a later date.

If you feel unwell at any point during or after the study you will be asked to stop and be supported in receiving any necessary medical attention.

You will be followed up for several weeks including with blood tests, although it is possible your follow-up could be up to 6 months.

There is a small theoretical risk of a reoccurrence of Leishmaniasis in individuals who develop any future immune problems (for example HIV and conditions requiring immune-suppressing medication). However, this is exceedingly rare in the species we are using in this study (*Leishmania major*).

What are the potential benefits of taking part?

The information from this study will help to further understanding of sand fly bites and contribute to developing a model for testing vaccines using infected sand fly bites. Ultimately this research will help in the effort to eradicate leishmaniasis.

It has been shown that people who have been exposed to leishmaniasis may develop a degree of immunity. This may provide protection against future *Leishmania* infections.

Do I have to take part in the infected biting study?

No, participation is optional. If you do decide to take part, you will be given a copy of this information sheet for your records and will be asked to complete a participant consent form. If you change your mind at any point during the study, you will be able to withdraw your participation without having to provide a reason.

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Figure 7.8: Proposed participant information leaflet for future infected sand fly biting study – for use by participant involvement group only

Parameter	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Extreme
Haematology				
Haemoglobin	10.0-10.9 g/dL	9.0-9.9 g/dL	7.0-8.9 g/dL	<7.0g/L
White Blood Count Upper	13.0–14.9x10^9/L	15.0-19.9 x10^9/L	20.0-29.9 x10^9/L	>30.0 x10^9/L
White Blood Count Lower	1.3-1.0 x10^9/L	< 1.0- ≥0.75 x10^9/L	<0.75- ≥0.5 x10^9/L	< 0.5 x10^9/L
Neutrophils	1.0 - 1.3 x10^9/L	0.75-0.999x10^9/L	0.5-0.749 x10^9/L	< 0.5 x10^9/L
Platelets	100.0-24.9 x10^9/l	50. - 99.9 x10^9/l	25.0 - 49.9 x10^9/l	< 25.0 x10^9/l
Biochemistry				
Potassium Hyperkalaemia Hypokalaemia	 5.6 – 6.0 mmol/L 3.0 - 3.4 mmol/L	 6.1 - 6.5 mmol/L 2.5 – 2.9 mmol/L	 6.6 – 7.0 mmol/L 2.0 - 2.4 mmol/L	 > 7.0 mmol/L < 2.0 mmol/L
Sodium Hypernatraemia Hyponatraemia	 146 - 150 mmol/L 130 - 135 mmol/L	 151 - 154 mmol/L 125 - 129 mmol/L	 155 - 159 mmol/L 121 – 124 mmol/L	 >159 mmol/L < 121 mmol/L
Bilirubin	1.25 - 2.0 x ULN	>2.0 - 2.5 x ULN	> 2.5 - 5 x ULN	> 5 x ULN
AST	1.25 - 2.5 x ULN	> 2.5 - 5.0 x ULN	> 5.0 - 10.0 x ULN	> 10 x ULN
ALT	1.25 - 2.5 x ULN	> 2.5 - 5.0 x ULN	> 5.0-10.0 x ULN	> 10 x ULN
Alkaline Phosphatase	1.25 - 2.5 x ULN	> 2.5 - 5.0 x ULN	> 5.0-10.0 x ULN	> 10 x ULN
Creatinine	1.1 – 1.3 x ULN	> 1.4 – 1.8 x ULN	> 1.9 – 3.4 x ULN	> 3.4 x ULN

Table 7.1: Laboratory reference ranges for FLYBITE study

Table of themes, with quotations from Public Involvement group excercise

Overarching Theme	Sub-theme	Sub-theme	Sub-theme	Quotation
1. Recruitment methods	1.1 Staff bulletin			"I found out [about the trial] through the staff bulletin 'cos I read that when it comes through on Thursday at work and I saw it in there and it sounded interesting." (P3, FG1)
	1.2 Study poster			"I spotted the poster thinking back and thought that looks interesting and then the process was much the same after that but yeah I saw the poster and that's how I got involved." (P6, FG1)
		1.2.1 Important to show financial compensation		"it's important to say you on the poster that you will be compensated for the time you spend, I don't think there's a need to put a number on it." (P5, FG1)
		1.2.2 Use of 'biting chamber' photo		"what appealed to me about the poster was that you actually showed the biting chamber because I think that was really useful because otherwise I would have thought that I'd have perhaps been in a room being bitten by flies" (P10, FG1)
	1.3 Local Link			"I actually saw the advertisement in the Local Link which is a free magazine that people in the area receive." (P16, FG2)
		1.3.1 Almost went unnoticed		"there was nothing particularly about it that, other than the fact that I knew what it was about. Had I not been interested or had ever done scientific research before I probably wouldn't have noticed it" (P15, FG2) "It looked exactly like a job advert." (P15, FG2)
		1.3.2 Unaware of article		"P2: I was interested to know if you'd linked up that there was an article and the advert. P16: No.

				P15: I don't think so. But I suppose that's fine because some people that read the whole local link would have read the article and then looked for the ad and then some people wouldn't have done and seen the ad anyway." (FG2)
		1.3.3 Perceived as 'junk mail'		"it's not that kind of popular is it as like a go to thing it does come through a lot of people's letter boxes as junk mail but yeah it will be seen as junk mail by a lot of people" (P15, FG2)
	1.4 Directly approached			"I was working in Q Block on the heating system and they said "would you like to do this?" and I said "yes" and that was it." (P7, FG1)
	1.5 Word of mouth			"somebody had told my wife and my wife told me and me and my wife was going for it. Unfortunately she wasn't successful due to health reasons but then I've been on the, I've been on it so it was all word of mouth." (P13, FG2)
	1.6 Overall suggestions for improvement	1.6.1 Using verbal methods		"some people are more easily persuaded by verbal means than written because we do live in a time of like too much information syndrome […] when everyone's presenting you with so much information I think on some level you just dismiss all of it." (P7, FG1)
		1.6.2 Emphasise end goal		"it [the study poster] didn't really sell what you wanted to do because the research it wants to help people all over the world that are affected with this fly. I think if they'd have focussed on actually people that want to save the world and help out it might have got more people in." (P14, FG2) local flyers
		1.6.3 Written content less vague / more eye-catching		"had I not been sat in front of it [study poster] was probably not as eye catching as it might have been." (P6, FG1)

				"it definitely could have been more eye catching. If you wanted more people to respond and it need to have more prominence then yeah you could have done with a bigger word, a picture you know, something, a slogan" (P15, FG2)
		1.6.4 Professional design support		"it's [i.e. study poster] the sort of thing you want like an advertising executive to design. Something that will really jump out to the lay person" (P7, FG1)
		1.6.5 Alternative methods suggested		"maybe you should do like a Facebook ad, I don't know, something on social media." (P15, FG2) "doing a little promotion video about the issue." (P15, FG2) "you could do on Minster Fm or Radio York would probably do an interview someone about it if you tell them about it." (P15, FG2) "local flyers and posters" (P13, FG2)
	1.7 Study website	1.7.1 Clear and informative		"it [the study website] was good, it was clear. I can't remember a lot about it other than the image and that it gave me the information I needed." (P3, FG1)
		1.7.2 Unaware of website		"I can't remember seeing the website, the link or anything. I don't recall…" (P16, FG2)
		1.7.3 Only used for recruitment		**"did you just go on [to the study website] at the point of wanting to take part in the project?** P5: Only when I was looking to take part. P (several): Yeah. **I: So you didn't use it as a resource beyond that?** P (several): No." (FG2)

		1.7.4 Reasons for not using website		"P6: I didn't think it was necessary. There was enough communication with Viv and everyone else to not need to use the website again. P10: Yeah we'd plenty of opportunity to ask questions to the people we were dealing with didn't we. P3: Yeah I agree." (FG1)
		1.7.5 Interested to access now aware		"I would be interested to know what's on it now. Does it have examples of other studies going on you know sort of globally Leish challenge or are there stories on it that tells you about people that are working in the countries with the victims or you know it would be interesting to know whether it, how much of a story it tells." (P15, FG2)
		1.7.6 Suggestions for improvement		"If I'd have seen it electronically I would have clicked on it. You know what I mean. I wouldn't have gone and typed it into Google." (P17, FG2)
2. Quality of participant-facing information	2.1 Well-received			"I think the [study information] sheets were completely adequate." (P15, FG2)
	2.2 More info re: extent of medical examination needed			"P10: it may well have been I didn't read it closely enough. That could be quite- P7: Yeah Viv did say to me. P10: I knew I was going to have a medical. P7: A full medical. P6: No I agree I didn't appreciate it would be quite as extensive." (FG1)
	2.3 Extensive medical - surprised but valued			"I thought it was quite nice. I know I'm healthy. I just didn't realise that was going to be as extensive as it was." (P6, FG1)

	2.4 Expressed interest in study outcomes			"I would be intrigued to see the progress of the research and where it goes and how successful it becomes…" (P14, FG2)
	2.5 Information needed for future study			"it [the future study] will take a lot more information to process in terms of I don't know what are the symptoms of the disease, what are the treatments, the potential sequels of either of those so it's a, I would think it's quite different" (P5, FG1)
3. Arrival process	3.1 Well-informed re: travel and parking			"it was very helpful the way they described how to, you know I said I'd be coming on a bicycle over Walmgate stray and they sort of told me exactly where I needed to go" (P16, FG2)
	3.2 Problem with Google Maps			"P14: I used Google maps so it took me to the other campus but then Nicky met me- P13: I did that two weeks previously." (FG2)
	3.3 Access to parking			"parking here was no hassle at all so it was very, very straightforward that for me and that made all the difference when I don't have to come onto campus and find a parking space and like you know I wouldn't have done it because I'd have just thought this is just going to be such a headache for me to do this." (P3, FG1)
	3.4 Having to wait outside if early	3.4.1 In the cold		"a couple of times I arrived early at the lab bit and I'd cycled and it wasn't very nice weather but I had to kind of stand outside and wait until the exact time to be admitted into the building." (P16, FG2) "they had problems taking my temperature because it was so cold I had to warm up. So if

				you were to sit inside you might have warmed up a bit quicker." (P13, FG2)
		3.4.2 Unaware of reception		"[you could] let people know that you can just go round the corner to the main entrance of Biology and there's a lovely big lounge area that you can sit in" (P15, FG2)
		3.4.3 Suggested doorbell		"they did say that for the next one [i.e. study] they were going to get a doorbell so you could ring in." (P13, FG2)
4. Screening process	4.1 Beneficial and accepted			"you're part of the study and that it's part and parcel of it so you get weighed and everything so you just accept it" (P16, FG2)
	4.2 Uncovered underlying health conditions			"I had the suspicion of a hernia but I was sort of putting it off until I told Dr Viv about it and he investigated and said yeah, it is a hernia and you need to get it sorted properly so yeah it was very beneficial." (P13, FG2)
	4.3 Study team were accommodating			"I know Nicky had to make quite an effort to come in earlier and I appreciated that." (P10, FG1)
	4.4 Experience of specific tests	4.4.1 Blood tests	Problems	"the first time I had blood taken they couldn't get any blood out of one arm and so they had to do the other and that arm got quite bruised and was quite sore and it was quite, there was quite a lot of discomfort. The other subsequent visits were fine and it was very painless" (P3, FG1)
			Fine and painless	"P16: I didn't have any problems with the blood tests, no. P13: No, absolutely fine." (FG2)

		4.4.2 Disliked weighing		“I didn’t like being weighed.” (P3, FG1)
		4.4.3 Pregnancy testing	Anxiety surrounding	“it’s vanishingly unlikely I could be pregnant there’s still a certain anxiety associated with having a pregnancy test to me.” (P10, FG1)
			Grateful	“very grateful for the pregnancy test, thank you.” (P15, FG2)
			Preference to take urine sample pots home	“for me it would have been easier to have a pot to take home.” (P10, FG1)
		4.4.4 Height measurement superfluous		“it takes almost no time [to take height measurement] but it’s like it’s everyone’s time writing it down, it’s a section on the form I mean it’s just a waste of resources. But then most of them make sense I mean they are taking I mean I don’t know the blood test makes sense, the temperature, the weight, other stuff makes sense I think only one is a bit kind of weird is the height.”
5. Experience of being bitten	5.1 A positive experience overall			“It was just a really nice experience. For me I mean, you know, I just found it all mod cons and everything was very comfortably ran because you just sit in a chair and back and yeah it was really nice, you know for my needs it was above and beyond what I needed.” (P7, FG1)
	5.2 Perception of the flies	5.2.1 Smaller than anticipated		“P14: They were quite small yeah. I was expecting a bit bigger. P15: Because you couldn’t really see them.” (FG2)
		5.2.2 Expecting more flies / bites		“I was expecting more flies in there than just five. Not knowing what was going to be happening but I just expected more bites.” (P13, FG2)

	5.3 "Unremarkable" – everything as expected			"It [the bite] was unremarkable to the point where I forgot I was taking part in a medical study." (P7, FG1)
	5.4 Physical side-effects	5.4.1 Skin reaction less than anticipated		"[The bite itself] was really minor, much less, I mean just a tiny red mark and I was expecting you know a kind of a horrible itchy lump so it was much less than I expected." (P12, FG1)
		5.4.2 Not painful		"I only got bitten properly once and I could just feel the slightest sort of prickling, nothing painful." (P10, FG1)
		5.4.3 Blistering		"It was very raised and rounded, it was like a small blister." (P13, FG1)
		5.4.4 Itchiness - needed antihistamines		"[The bite] was just itchy so the more I itched it and then it got the skin slightly torn and then just looked like an insect bite, just a scratched insect bite that I would itch which made it worse." (P15, FG2) "I did get the antihistamines on my final visit just so that I could take some when it was itchy." (P15, FG2)
		5.4.5 No medication needed		"Just didn't need to [take antihistamines]" (P13, FG2)
		5.4.6 Glad of reaction		"I was happy in a way that I did have a slight reaction that something was happening." (P13, FG2)
	5.5 Curiosity as flies began to bite			"Slightly curious, really just like ok let's do science here. Strap it on and off we go […] You've been bitten by and insect before, you're going to volunteer to be bitten by seven now, let's see what it does." (P7, FG1)

	5.6 Excitement as flies began to bite			“Really excited when they started biting.” (P10, FG1)
	5.7 Pre-bite anxiety			“I was slightly anxious that morning because I thought, I know it’s only a small area but I was thinking if they really really bite, I mean I come up quite badly with mosquito bites” (P10, FG1)
	5.8 Bite procedure	5.8.1 Longer than anticipated		“the actual bite itself was about three hours in total with the half an hour of biting and then all of the monitoring stuff which did take out the afternoon.” (P6, FG1)
		5.8.2 Suggestions for improving trial environment	Comfortable chair	“You could have had a slightly more comfy chair to sit in. […] Because I was sort of sideways to the desk and you were stretching your arm out for quite a long time.” (P10, FG1)
			Refreshments	“it would have been nice to have been able to maybe make a cup of tea or coffee in that because it was quite a long time.” (P16, FG2)
			Entertainment	“I think the option perhaps for somebody to watch a film or, you know and then just be monitored in between, might be helpful for some people.” (P10, FG1)
			Use of non-clinical environment	“[After the bite procedure] I thought you’d then get to sit in like a lounge area rather than the same clinical room, yeah. It didn’t matter at all but that’s just what I had in my head. That those three hours would be a little bit more comfortable in a way.” (P15, FG2)
		5.8.3 Well-informed		“they explained it really well as well so they explained the procedure “Oh there’s a fly bitten you and you can see it

				sucking up the blood now" (P14, FG2)
	5.9 Post-bite diary	5.9.1 Paper-diary fine		"Well I work better with paper. I'm still a dinosaur when it comes to all the sort of technology and everything so for me it was fine to have to you know didn't forget and took it with me and everything so yeah." (P16, FG2)
		5.9.2 Suggestions for future		"if you really wanted to bother like an app for you to quickly fill in your diary every day would have been amazing." (P15, FG2)
	5.10 Aftercare	5.10.1 Confident re: how to contact team		"If I'd needed to get help or something I felt quite confident that I would have been able to." (P17, FG2)
		5.10.2 Could not remember advice given		"I can't remember what the advice was." (P15, FG2)
		5.10.3 Good support network		"it seemed that everything was in place fine." (P13, FG2)
6. Overall study experience	6.1 Described as positive			"it was sort of a privilege to be a part of but like you say Viv had explained everything so well from what I say paperwork I never visited the website but anyway it was so clear there was not one point in the entire process for me was there any surprises it was exactly as sold and you know you just did it and it was fine, no surprises, no downsides, for me I mean it was a positive pleasant experience. I didn't, no apprehension at any point, there was no worry I mean I suppose I was just so well informed, due credit to them really." (P7, FG1)
	6.2 Well-informed re: entire study			"they [the team] made sure you understood especially the first ones they would constantly ask you do you have another question and kind of repeat things just to

				make sure you didn't miss anything which I think is really good." (P5, FG1)
	6.3 The study team	6.3.1 Professional		"it [involvement with the study and study team] was the most professional thing that's happened to me in a long time." (P7, FG1)
		6.3.2 Good communication		"I was expecting a phone call and I got a phone call from Viv I think it was and it was all quite seamless I felt and I think when you sign up for something you want someone to respond and you don't want to feel like you've made the effort to send an email and no one's replied for ages but it was a good follow up I think." (P3, FG1)
		6.3.3. Providing opportunities to ask questions		"we'd plenty of opportunity to ask questions to the people we were dealing with" (P10, FG1)
		6.3.4 "Warm"		"I'd applied for scientific research before but it wasn't as like, as warm as this one." (P14, FG2)
		6.3.5 Enjoyed social interaction with		"they [the team] always had lots to talk about and so it was more enjoyable rather than being with someone who's quite straight edge and quite strict and you know not really open to talk to or you know that kind of person so yeah that was quite pleasant." (P14, FG2)
		6.3.6 Good relationship with		"you build up a bit of a relationship [with the team] don't you, you get to know them, have these chats with them and it was really pleasant. They were waiting for me every time I arrived there." (P3, FG1)
	6.4 No safety concerns			**"Did you have any safety concerns at all when you**

				were part of the project? The trial. P (Several): No. **I: None at all?** P (Several): No." (FG1)
	6.5 Felt appreciated, valued, well cared for			"It was one of the nicest things about it that you felt that you were all involved in something interesting that they were genuinely excited about and that they appreciated your time and volunteering so that was really nice." (P10, FG1) "we were treated very well especially as I say when the notice about the high blood pressure and Dr Viv was like you need to get it checked throughout the visit he was quite, he seemed genuinely concerned about it which was nice, he wasn't just treating you like a number he was treating like a person and taking care of you." (P13, FG2)
	6.6 Time commitment	6.6.1 More than anticipated		"for the three hours I took a half day holiday and for this I'm trading time in lieu that I worked over Christmas so that's a commitment that I've had to make, which is fine but time commitment is an issue for most people I think." (P10, FG1)
		6.6.2 Grateful for flexibility offered		"they [the team] were prepared to be flexible with the times for you to make it as convenient as possible, the time to come and meet with them so that helps a lot too." (P5, FG1)
		6.6.3 Virtual follow-up versus in-person contact		"if it [taking bite photos] was beneficial to the study then I wouldn't mind doing it." (P13, FG2)

				"you realise that whatever was going on was perfectly normal but you know had there been some sort of reaction or whatever you would like to think that you were being seen and they would pick something up or whatever it was because if you hadn't shown the bite you might think it was perfectly normal for it do something." (P17, FG2)
	6.7 Thoughts on level of remuneration	6.7.1 A satisfactory amount, about right		it [the level of remuneration] was about right for the time commitment and for the amount of blood." (P10, FG1)
		6.7.2 Would have taken part for less		"I'd have done it for a lot less. I didn't, although I did it primarily for money I suppose I would have been perfectly happy well with sort of twenty quid…" (P10, FG1)
		6.7.3 Money a bonus, secondary		"it was secondary for me, it was very useful for me as I don't earn at the moment it's very nice to have that bit of extra cash but I actually was just really interested in the project." (P6, FG1)
		6.7.4 Money as key motivator		"it was a very interesting study but I think the main reason was I thought, ooh here's a study I can get paid for and raise some money." (P10, FG1)
		6.7.5 Importance of versus not relevant		"getting some compensation makes you feel like your time is valuable, you know I have very little free time and to do it for nothing and take a lot of time off I would be reluctant to do that […] I don't think everyone should have to do it for free, if you'd like to it's not a bad thing to get a bit of money as part of it." (P3, FG1)

				"it [the remuneration] wasn't relevant, I actually waivered my compensation so I don't. I mean I checked it the time commitment was doable as I work on campus and I have flexible hours I could work around it and ok it looks like I can do it so I went for it, I didn't really consider it." (P5, FG1)
	6.8 Reasons for taking part	6.8.1 Interest in condition/research		"I'd heard about the condition before but also I've taught, you know having taught biology for a long time and sort of talked about taking part in scientific research and I thought well you know how about me doing it myself you know so it was just an interest really" (P17,FG2)
		6.8.2 Trial meaningful and worthwhile		"It was just nice to have done something maybe a bit meaningful for a change…" (P7, FG1)
		6.8.3 Desire to help others		"it's my chance to sort of contribute to some sort of research which in the long run is going to hopefully benefit quite a few people." (P16, FG2)
	6.9 Considerations for future study			"I think it [the study] needs to be promoted more for what the end goal is rather than the actual process of it cos then I think more people like myself would be intrigued because they want to help things, they enjoy research, they enjoy helping things" (P14, FG2)

Table 7.2: Public involvement group table of themes

Produced by VP, NM and GJ.

FLYBITE Clinical Study Team
Translational Research Facility (Q Block)
Hull York Medical School
Department of Biology
University of York
Wentworth Way
York, YO10 5DD, UK

Direct Telephone: +44 (0) 1904 328934
Direct Facsimile: +44 (0) 1904 328844
E-Mail: vivak.parkash@york.ac.uk

A clinical study to develop a sand fly biting protocol using pathogen-free blood-fed sand flies – Pre-screening questionnaire

Volunteer Name _____

DOB (dd/mm/yyyy) _____

Email address _____

Contact telephone number _____

Please respond with Y / N / NK (Yes, No, Not known)

To the best of my knowledge:		Response
1	I confirm that I **am** aged between 18 – 65 years old	
2	I confirm that I **will** be available for the duration of the study	
3	I confirm that I have **no** history of *Leishmania* infection	
4	I confirm that I have **no** history of travel within the last 30 days to regions where *Leishmania major*-transmitting sand flies are present*	
5	I confirm that I have **no** history of more than 30 days continuous stay in regions where *Leishmania major*-transmitting sand flies are present*	
5	I confirm that I have **no** current chronic illness requiring hospital specialist input or any other significant ongoing medical condition	

*This refers to regions where *Leishmania major*-transmitting sand flies are endemic including (but not limited to) the Middle East, Sub-Saharan Africa, and Asia

Please detail any allergies and the nature of the allergy:

……

……

……

Name of potential participant _____ Date _____ Signature _____

Any information contained within this questionnaire will not be retained and will be destroyed if you do not proceed with the study

FLYBITE pre-screening questionnaire v0.1 26/06/2019

Figure 7.9: FLYBITE study pre-screening questionnaire

VOLUNTEERS WANTED

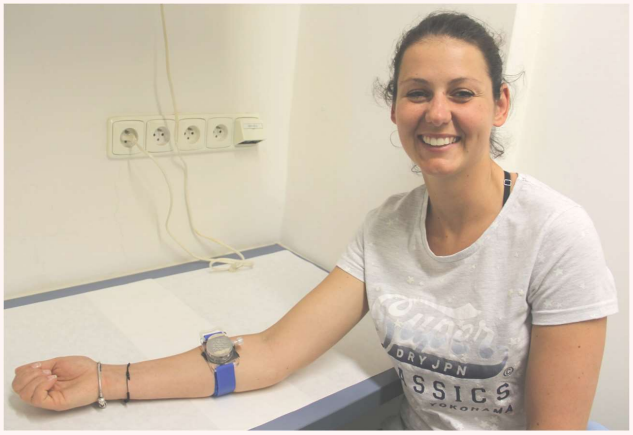
FOR HUMAN CHALLENGE STUDY

The Department of Biology at the University of York,
are looking for volunteers aged 18-65
to take part in a sand fly biting study

The results of the study will help in the development
& testing of vaccines for leishmaniasis, a disease which affects the skin

Volunteers will be reimbursed for their time and expenses

A volunteer wearing a biting chamber with sand flies inside



Interested in volunteering?
Contact the project team at:
liz.greensted@york.ac.uk, or vivak.parkash@york.ac.uk
T: 01904 328621

For further information: https://leishchallenge.org

LEISH Challenge

HULL YORK MEDICAL SCHOOL

UNIVERSITY of York

Advertisement for FLYBITE v1.0 17/05/19

Figure 7.10: FLYBITE study recruitment poster

LEISH CHALLENGE:
UNINFECTED SAND FLY BITING STUDY

Participant Information Leaflet

For more information contact the project team at:
liz.greensted@york.ac.uk, or vivak.parkash@york.ac.uk,
01904 328621

Background

The University of York would like to invite you to take part in a research study, "A clinical study to develop a sand fly biting protocol using pathogen-free blood-fed sand flies".

Our research relates to an infection called leishmaniasis. The disease leishmaniasis mainly occurs in hot and tropical countries, affects millions of people and causes around 20,000 deaths across the world every year. There are different types of leishmaniasis around the world and some can be very serious. They affect the skin (cutaneous leishmaniasis) or the internal organs of the body (visceral leishmaniasis). Some of the milder forms will produce skin problems which will be localised, whilst other forms of leishmaniasis will cause widespread skin changes. The skin lesions of cutaneous leishmaniasis can be disfiguring if left untreated. These can cause stigma, and disproportionately affect children. We do have some treatments for leishmaniasis but many of them are not easy to use or don't work well. Therefore, we need new treatments and would like to find vaccines that prevent or work against leishmaniasis.

A solution being adopted for other diseases, which we now wish to adopt for leishmaniasis is to develop a 'human challenge model' also called a 'Controlled human infection model' (CHIM). These models involve deliberate exposure of individuals to an infection, in order to better understand how the disease works and to test potential vaccines and treatments. CHIM studies have been refined in the last 20 years. They have contributed vital scientific knowledge that has led to advances in the development of drugs and vaccines. Current diseases for which human challenge models are available include malaria, dengue fever, pneumonia, cholera, and influenza.

What is the purpose of the study?

Leishmaniasis is caused by the Leishmania parasite and is transmitted by sand flies. The parasite is tiny and not visible to the naked eye, whereas the particular sand fly is visible but small and inconspicuous. This study is simply looking at how we can replicate a sand fly bite, the same as in the wild, in a safe and consistent manner.

This is an initial study using uninfected (disease-free) sand flies. The information from this study will help us to develop a model in the future using infected sand flies so that we can assess any future vaccines against Leishmaniasis. We will not be testing a vaccine in this study.

Before agreeing to take part in this research, please read this information sheet carefully and let us know if anything is unclear or you would like further information.

We will also ask you to contribute to a focus group after the sand fly biting study to explore your experiences of taking part in this study and gather your views on how the rest of the study should take place. With your permission, we would like to record the focus group meeting.

Why have I been asked to take part?

We are advertising for volunteers to take part in this study. Volunteers must be men and women aged between 18 and 65 years old. Enrolled female participants must not be pregnant or breastfeeding, and must be using a reliable form of contraception.
Volunteers must be healthy and not be at risk of serious infections and must not have any chronic skin conditions. Volunteers should not have travelled in the last 30 days to any area where leishmaniasis is present or for 30 consecutive days at any time in the past. There should be no history of significant reaction to insect bites.

Where do the sand flies come from?

We are working with scientists in the Czech Republic who are 'insect experts' (entomologists). They have been rearing sand flies for several years and are experts in producing disease-free sand flies for use in research. We have been working closely with these scientists to produce the right kind of sand fly for our study. There will be 2 different types of sand flies used, both of which have been shown to transmit *Leishmania*.

What will happen if I agree to take part?

This study is designed to test safety and assess the sand fly biting protocol in healthy volunteers.

- There will be 5 study visits in total including screening, each lasting between 30 minutes to 3 hours. These will take place this will take place at the Translational Research Facility (Q Block), HYMS / Department of Biology, University of York, York, YO10 5DD.
- In addition to the 5 study visits, we would also like you to take part in a focus group. This will take place after the last study visit and it is anticipated that this will last between 2-3 hours and involve all the participants that have taken part in the study. The focus group will provide the opportunity for you to share your experiences of participating in the study and help us to shape the design of the next stage of the research. This will take place at the University of York, York, YO10 5DD.
- Study visit 1: Screening blood tests will be performed to assess suitability. This is likely to take place a few weeks before the main study.
- Study visit 2: The 2nd visit will be the biting visit itself. Using a custom wrist watch-like biting chamber, up to 5 uninfected sand flies will be placed against the volunteers' skin.
- The exposure will be for up to 30 minutes.
- Blood tests will be performed following the study to help assess safety aspects.
- Photographs and videography of the bite site will be performed with your permission.
- The volunteers will be observed for a period of time before being allowed to go home. A doctor, nurse and an insect specialist (entomologist) and other senior study investigators will be present throughout.
- For any local skin reactions, antihistamine medications and creams will be offered.
- Follow-up visits will take place at approximately 3 days, 10 days and 21 days after the biting study. (Study visit 3 to 5). These will each be 30 minute visits for blood tests and examination of the bite site.
- Following the study, non-identifiable information will be recorded and will contribute to future publication and research.

You will be remunerated for your time and for travel expenditure. You will be contacted by the study team who will discuss the study in further detail including asking further questions to confirm eligibility. We will ask your permission to contact your GP to confirm your eligibility prior to starting the study.

Who is organising and funding this study?

Funding has been provided by the Medical Research Council and Department for International Development (Ref: MR/R014973/1)

What are the potential risks of taking part?

Volunteers may experience a 'pin-prick' sensation when bitten by sand flies. Common effects following sand fly bites (as with all insect bites) include some swelling, pain and redness at the bite site. This may take up to 48 hours to develop. Just as with any insect bite there is the potential for a skin infection which may require antibiotic treatment.

The species of sand fly that we are using has not been associated with any serious reaction in the past. Although hundreds of thousands of people are bitten by sand flies every day without any serious effects, we want to absolutely ensure your safety. For this reason blood tests taken prior to the study will help to identify volunteers who are more likely to suffer adverse effects and these individuals will be excluded from participating further (this includes a blood test to look specifically at sand fly bite reaction as well as other reactions).

To-date, there have been no reported cases of anaphylactic (life-threatening) reactions to sand fly bites in the whole medical literature. However, there might still be a small theoretical risk of serious reaction to sand fly bite, just as with any insect bite. When a serious reaction occurs, although rare it usually presents with breathing problems and widespread skin rash. Within the very small number of people who develop a serious reaction, a small number of these may be at risk of death. We have appropriate treatment and a medical team on-site at all times in the very unlikely event that a serious reaction occurs.

Volunteers will undergo a blood test a few weeks before and shortly after the sand fly bite study. Further blood tests will take place on the follow-up visits. There is a small risk of bruising at the site from where the blood sample is taken.

If you feel unwell at any point during or after the study you will be asked to stop and be supported in receiving any necessary medical attention. This will include a support line for advice if needed and admission to hospital, although this is not thought to be likely.

What are the potential benefits of taking part?

The information from this study will help to further understanding of sand fly bites and contribute to developing a future model for testing vaccines using infected sand fly bites. Ultimately this research will help in the effort to beat leishmaniasis.

You will also undergo health screening by the medical doctors who are involved in the study. This will involve a history, physical examination and blood tests.

Do I have to take part in the uninfected biting study?

No, participation is optional. If you do decide to take part, you will be given a copy of this information sheet for your records and will be asked to complete a participant consent form. If you change your mind at any point during the study (including during the focus group), you will be able to withdraw your participation without having to provide a reason. Any samples and information obtained up until the point of withdrawal will be retained.

What will happen to my samples?

The blood samples will be tested at the Translational Research Facility, HYMS / Department of Biology, University of York and York Hospitals NHS Foundation Trust. This is where they will be studied by the research team. Samples will be tested in secure laboratory facilities accessible only by authorised research staff. Samples will be labelled with a unique ID number and the date only but no personal details, so they cannot be identifiable as having been donated by you. The samples will only be used in research projects that have been independently reviewed and approved by the Department of Biology Ethics Committee.

What happens if there is a problem?

If you feel at any stage that there has been a problem with your participation, you can discuss this matter, in confidence, with Professor Charles Lacey, the Investigator, on: tel 01904 328879 or email: charles.lacey@hyms.ac.uk

University of York holds Public Liability ("negligent harm") and Clinical Trial ("non-negligent harm") insurance policies which apply to this study. If you can demonstrate that you experienced serious and enduring harm as a result of your participation in this study, you may be eligible to claim compensation without having to prove that University of York is at fault. If the injury resulted from any procedure which is not part of the study, University of York will not be required to compensate you in this way. Your legal rights to claim compensation for injury where you can prove negligence are not affected. Please contact the Chief Investigator if you would like further information about the insurance arrangements which apply to the trial.

On what basis will you process my data (i.e. personal information)?

Under the General Data Protection Regulation (GDPR), the University has to identify a legal basis for processing personal data and, where appropriate, an additional condition for processing special category data. (Please see the additional document on data protection and personal information.

What happens next?

If you are interested in this study or if you have any questions, please contact one of the study team members (details given on the front of this leaflet). One of our study doctors will talk to you about what is involved and ask questions about your suitability for this study. The next step will then be to invite you to a screening assessment at our facilities at the University of York.

This project: "Development of a human challenge model of Leishmania major infection as a tool for assessing vaccines against leishmaniasis" is funded by the Medical Research Council and Department for International Development (ref: MR/R014973/1)

MRC Medical Research Council

Department for International Development

LEISH CHALLENGE:
UNINFECTED SAND FLY BITING STUDY

Participant Data Protection and Personal Data

On what basis will you process my data (i.e. personal information)?

Under the General Data Protection Regulation (GDPR), the University has to identify a legal basis for processing personal data and, where appropriate, an additional condition for processing special category data.

In line with our charter which states that we advance learning and knowledge by teaching and research, the University processes personal data for research purposes under Article 6 (1) (e) of the GDPR:

Processing is necessary for the performance of a task carried out in the public interest

Special category data is processed under Article 9 (2) (j):

Processing is necessary for archiving purposes in the public interest, or scientific and historical research purposes or statistical purposes

Research will only be undertaken where ethical approval has been obtained, where there is a clear public interest and where appropriate safeguards have been put in place to protect data.

In line with ethical expectations and in order to comply with common law duty of confidentiality, we will seek your consent to participate where appropriate. This consent will not, however, be our legal basis for processing your data under the GDPR.

How will you use my data?

Data will be processed for the purposes outlined in this information sheet.

Will you share my data with 3rd parties?

No. Data will be accessible to the project team at York only.

Anonymised data may be reused by the research team or other third parties for secondary research purposes.

How will you keep my data secure?

The University will put in place appropriate technical and organisational measures to protect your personal data and/or special category data. For the purposes of this project we will store data on dedicated servers and / or on the University of York central data store, which provided secure long term storage for data, including daily backups, according to the University of York Research Data Management Policy.

A study master file will be created to include the study protocol, original signed consent forms and ethics/governance documentation. It will also include a list of study group participants, their email

address and their telephone number. The study master file will be stored at Department of Biology in a secure, fire and rodent-proof cabinet that is only accessible to authorised members of staff.

Information will be treated confidentiality and shared on a need-to-know basis only. The University is committed to the principle of data protection by design and default and will collect the minimum amount of data necessary for the project. In addition, we will anonymise or pseudonymise data wherever possible.

Will you transfer my data internationally?

No. Data will be held within the European Economic Area in full compliance with data protection legislation.

Will I be identified in any research outputs?

No.

How long will you keep my data?

Data will be retained in line with legal requirements or where there is a business need. Retention timeframes will be determined in line with the University's Records Retention Schedule.

What rights do I have in relation to my data?

Under the GDPR, you have a general right of access to your data, a right to rectification, erasure, restriction, objection or portability. You also have a right to withdrawal. Please note, not all rights apply where data is processed purely for research purposes. For further information see, https://www.york.ac.uk/records-management/generaldataprotectionregulation/individualsrights/

Questions or concerns

If you have any questions about this participant information sheet or concerns about how your data is being processed, please contact Liz Greensted, Project Manager (liz.greensted@york.ac.uk), in the first instance. If you are still dissatisfied, please contact the University's Acting Data Protection Officer at dataprotection@york.ac.uk.

Right to complain

If you are unhappy with the way in which the University has handled your personal data, you have a right to complain to the Information Commissioner's Office. For information on reporting a concern to the Information Commissioner's Office, see www.ico.org.uk/concerns.

This project: "Development of a human challenge model of Leishmania major infection as a tool for assessing vaccines against leishmaniasis" is funded by the Medical Research Council and Department for International Development (ref: MR/R014973/1)

MRC Medical Research Council

Department for International Development

Figure 7.11: FLYBITE participant information leaflet

FLYBITE Clinical Study Team
Translational Research Facility (Q Block)
Hull York Medical School
Department of Biology
University of York
Wentworth Way
York, YO10 5DD, UK

Direct Telephone: +44 (0) 1904 328934
Direct Facsimile: +44 (0) 1904 328844
E-Mail: vivak.parkash@york.ac.uk

Participant consent form

A clinical study to develop a sand fly biting protocol using pathogen-free blood-fed sand flies

	I acknowledge by my initials that:	Please initial box to agree
1	I confirm that I have read and understood the Information Sheet and GDPR form dated …. for the above study and have had the opportunity to ask questions.	
2	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.	
3	I understand that sections of my study documents may be looked at by responsible individuals involved in the running of the trial or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my study documents and/or medical records.	
4	I agree to be entered onto a database (TOPS) as a measure to prevent over-volunteering and I agree to have a photograph taken for identification purposes.	
5	I understand that I will take part in a focus group, which will be audio recorded and analysed, but only identified in that by my study number	
6	I agree that the data from my participation may be used for additional related future research provided that research is given additional approval from a Research Ethics Committee.	
7	I agree to take part in the above study and to abide by the restrictions set out in the information sheet.	
8	I agree to allow anonymised photography and videography during the biting study and follow-up appointments, which may be used in publication.	

9	I agree that my GP can be contacted for information that may affect my participation in the trial and will be informed if I decide to take part. I give permission that my GP may be notified of health issues that may be identified through my participation if the study doctor considers this appropriate.	
10	I understand that samples of my tissue, including blood samples, will be taken for analysis. I agree to these samples being stored for the purpose of this study and used for future research provided that research is given additional approval from a Research Ethics Committee	

Name of volunteer	Date	Signature
_____	_____	_____
Chief Investigator or person designated by Chief Investigator	Date	Signature
_____	_____	_____

FLYBITE Volunteer consent form v1.0 14/05/2019

Figure 7.12: FLYBITE study participant consent form

FLYBITE Clinical Study Team
Translational Research Facility (Q Block)
Hull York Medical School
Department of Biology
University of York
Wentworth Way
York, YO10 5DD, UK

Direct Telephone: +44 (0) 1904 328934
Direct Facsimile: +44 (0) 1904 328844
E-Mail: vivak.parkash@york.ac.uk

GP address

Date

Dear Dr,

RE: A clinical study to develop a sand fly biting protocol using pathogen-free blood-fed sand flies

We are writing to you about *Volunteer Name (DOB dd/mm/yyyy)* who we understand is your patient and who has consented to be entered into the above trial.

Please find enclosed a volunteer information sheet and the eligibility criteria for this trial. In order for us to proceed with enrolling your patient into this study we are seeking confirmation from you, their GP that:

- they have not recently participated in any other clinical trials
- they have no significant ongoing illness
- that there is no reason that you feel your patient should not be entered into the study after review of the enclosed documentation

We therefore enclose a very short questionnaire for you to complete in order to confirm these details. We also enclose a copy of the signed consent form which indicates that the volunteer has provided consent for us to contact you to request this information.

We would be grateful if you could complete this form as soon as possible and return it either in the enclosed prepaid envelope or by fax to the number at the top of this letter. Upon receipt of the completed form we will send you an honorarium of £40.

We will keep you up to date with your patient's progress but you have any concerns or questions in the meantime please do not hesitate to contact the research team.

Yours sincerely,

Prof Charles JN Lacey
Chief Investigator

Figure 7.13: FLYBITE study letter to General Practitioner for suitability of participant enrolment

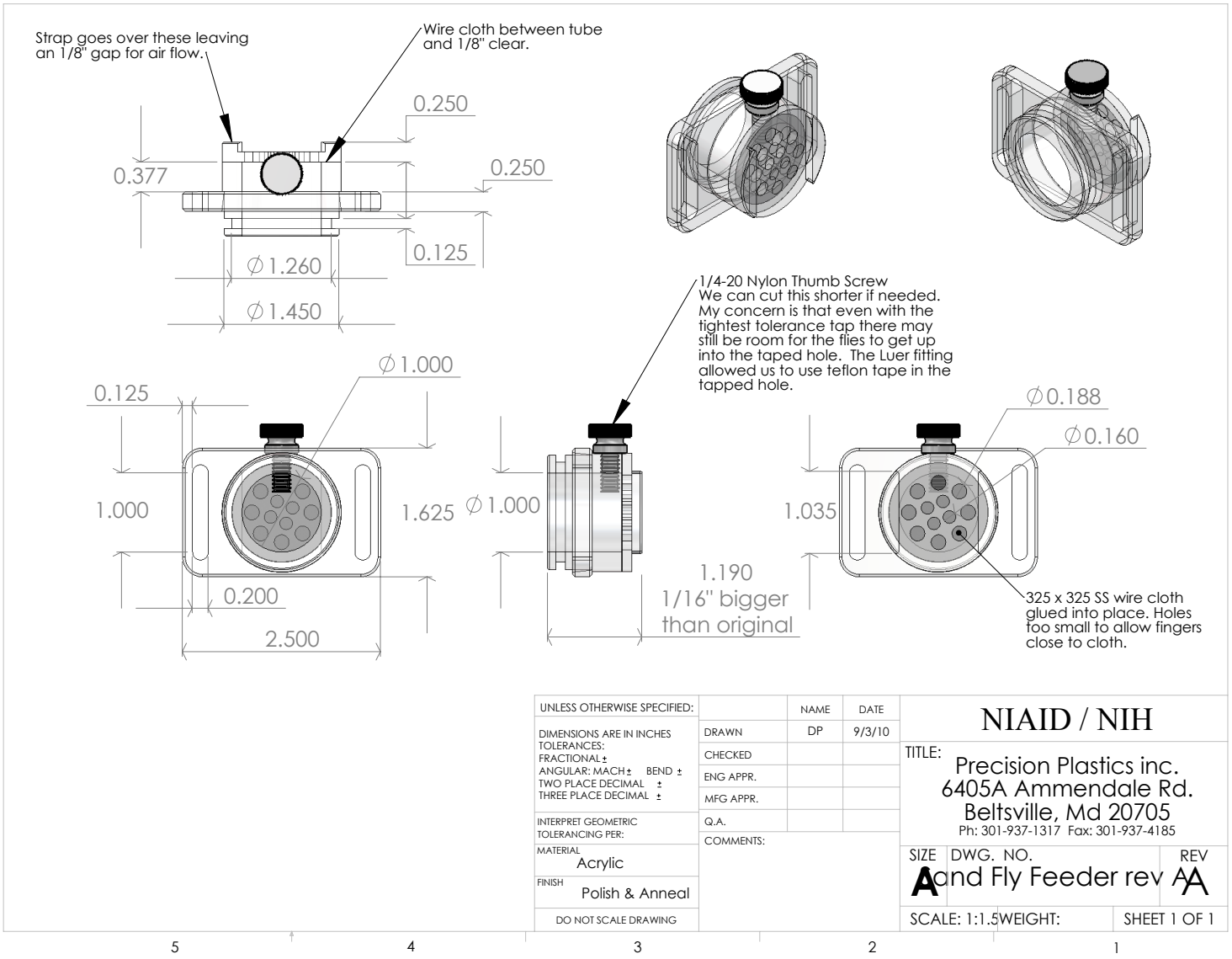


Figure 7.14: Sand fly biting chamber schematic

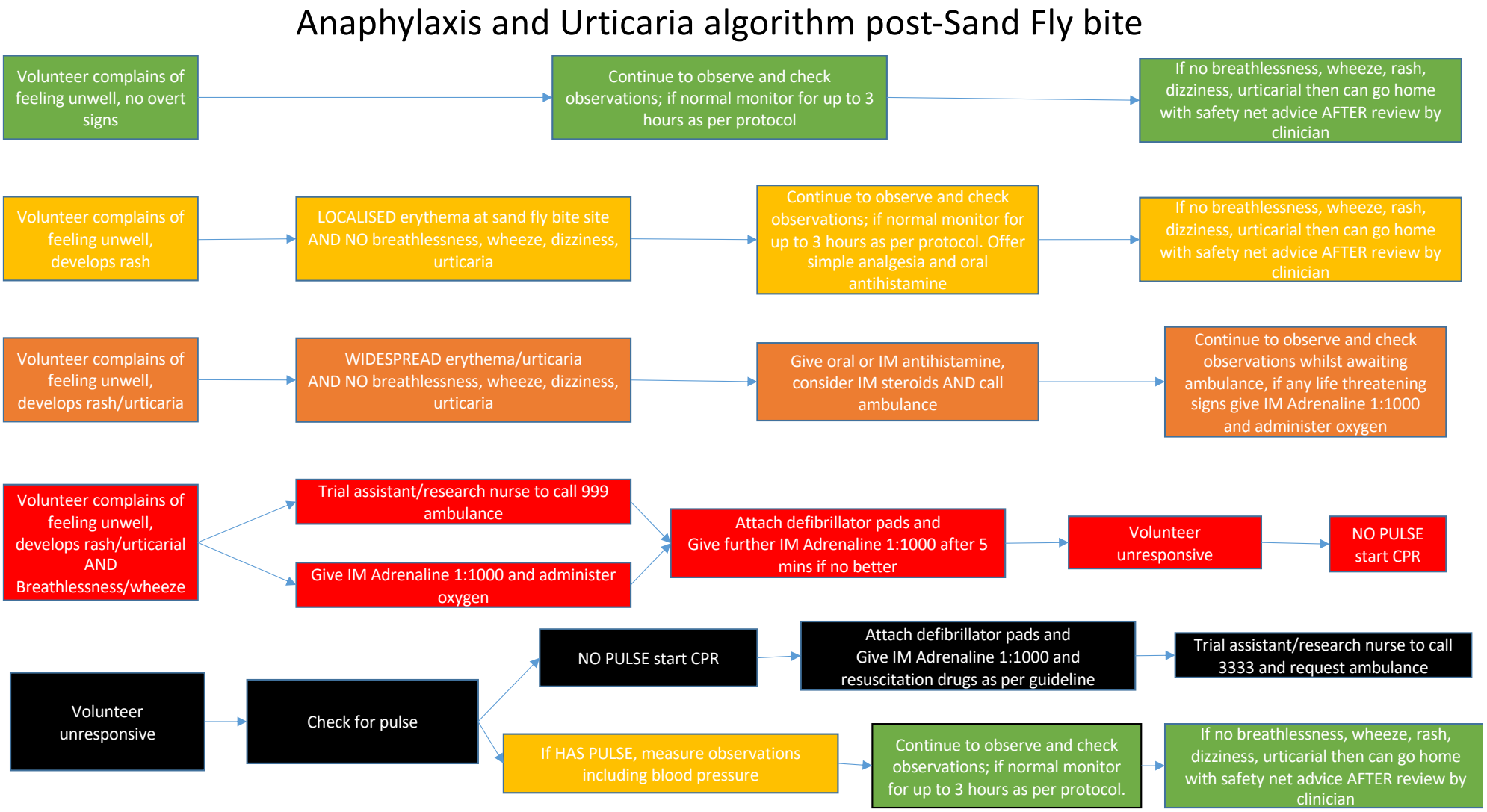


Figure 7.15: Emergency algorithm for FLYBITE study

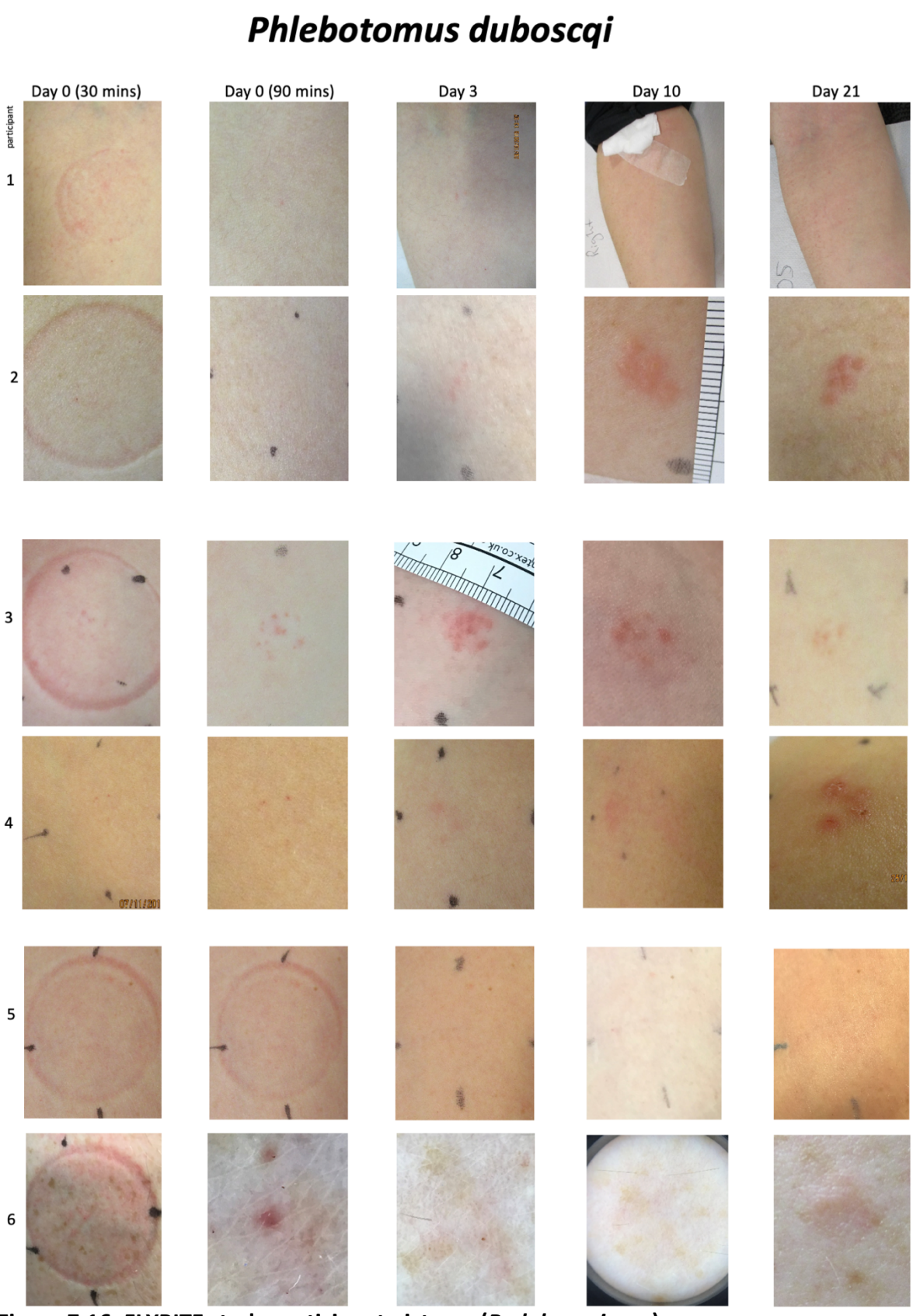


Figure 7.16: FLYBITE study participant pictures (*P. duboscqi* arm)

Dermoscopy and digital camera image also reproduced in (Parkash et al., 2021a).

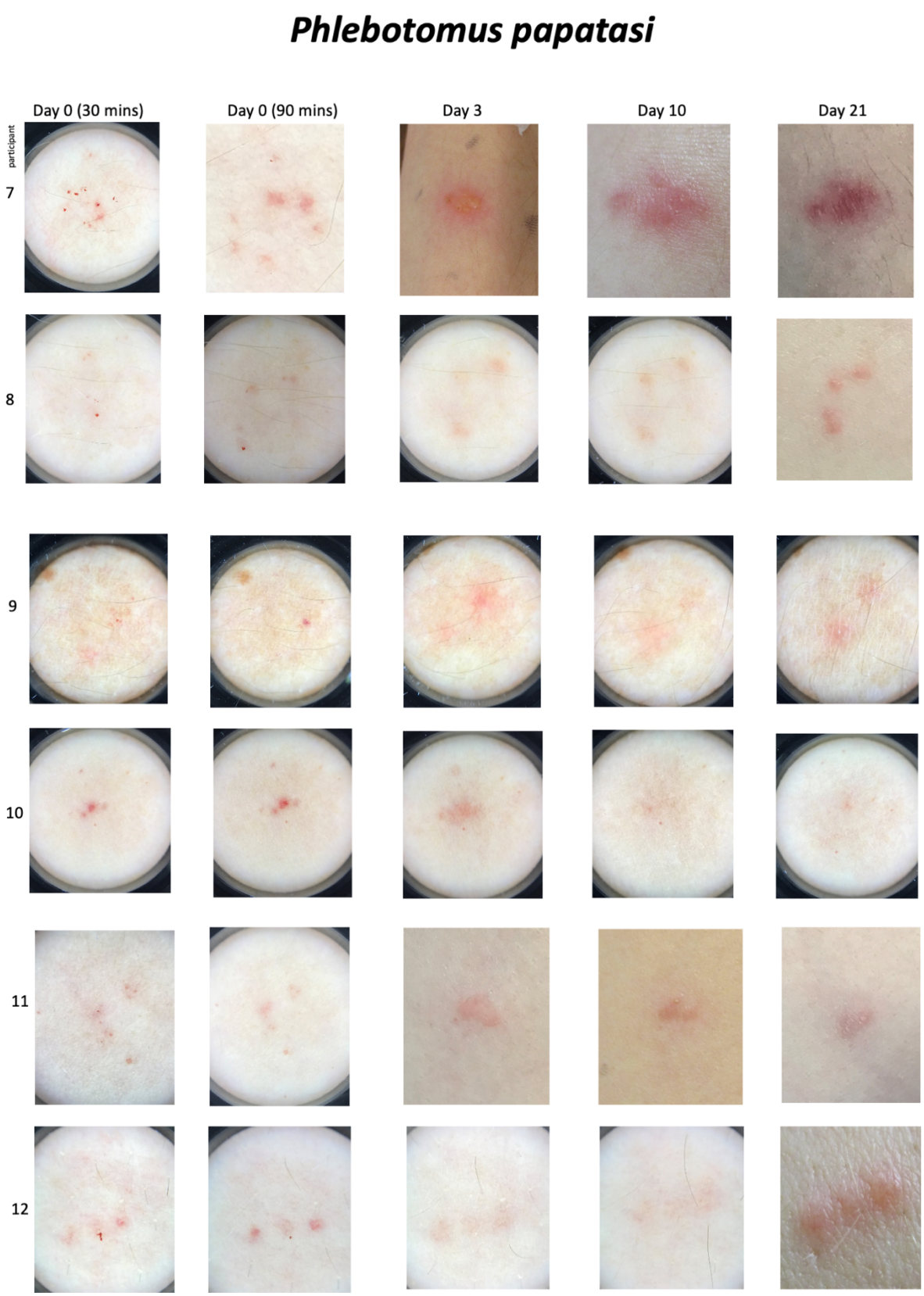


Figure 7.17: FLYBITE study participant pictures *(P. papatasi arm)*

Dermoscopy and digital camera image also reproduced in (Parkash et al., 2021a)**.**

Solicited study adverse reaction grading for FLYBITE

	Grade 1 (Mild)	**Grade 2 (Moderate)**	**Grade 3 (Severe)**	**Grade 4 (Extreme)**
GENERAL				
Fever Oral>12 hours	37.7 - 38.9°C (100.0 – 101.5°F)	39.0 – 39.7°C (101.6 – 102.9°F)	39.8 – 40.5°C (103 - 105°F)	>40.5°C (105°F) OR max temp of >105°F
Chills/rigors	Mild hot/cold flush requires blanket or occasional aspirin/paracetamol	Limiting daily activity >6 hours, or need regular aspirin/paracetamol	Uncontrollable shaking, treatment from doctor needed	Hospitalisation
Malaise/abnormal tiredness	Normal activity reduced – not bad enough to go to bed	Fatigue such that ½ day in bed for 1 or 2 days	Fatigue such that in bed all day or ½ day for more than 2 days	Hospitalisation
General (all over) muscle aches and pains (myalgia)	No limitation of activity	Muscle tenderness, aches/pains limiting activity e.g. difficulty climbing stairs	Severe limitation e.g. can't climb stairs	Hospitalisation
Headache	No treatment or responds to paracetamol like treatment	Regular paracetamol like treatment needed	Regular strong painkillers needed	Hospitalisation
Nausea	Intake maintained	Intake reduced less than 3 days	Minimal intake 3 days or more	Hospitalisation
Immediate general reactions (within 6 hours of sand fly bite)			Laryngeal oedema insufficient to require intubation; diarrhoea insufficient to require IV fluids, or asthma insufficient to require hospitalisation OR Urticaria, angiooedema	Anaphylactic shock
CUTANEOUS				

Itch / pruritus	Mild itching, not requiring specific therapy	Moderate itching, requiring intermittent antihistamines	Severe itching, requiring regular antihistamines	Generalised itching, requiring on going medical management
Discomfort/pain at the biting site	Mild pain that responds to paracetamol like treatment, if needed	Pain requiring regular paracetamol like treatment	Pain requiring regular strong painkillers	Hospitalisation
Erythema at the biting site	Mild erythema localised to within the outline of the biting chamber	Moderate erythema beyond the outline of the biting chamber but <30% of the arm circumference	Erythema greater than 30% of the arm circumference	Hospitalisation
Blistering or ulceration at biting site	Fluid filled vesicles or superficial disruption of epithelium covering an area < 1cm radius	Fluid filled vesicles or superficial disruption of epithelium, area 1 - 2cm radius	Full thickness disruption of epithelium	**Necrosis**
Swelling at biting site	Soft swelling – local Swelling <1 cm	Soft swelling – local Swelling 1-2 cms	Soft swelling – local Swelling > 2 cms	

Table 7.3: Solicited study adverse reaction grading for FLYBITE

FLYBITE study - Table of themes, with quotations.

Overarching Theme	Sub-theme	Sub-theme	Sub-theme	Quotation
1. Recruitment methods	1.1 Staff bulletin			"I found out [about the trial] through the staff bulletin 'cos I read that when it comes through on Thursday at work and I saw it in there and it sounded interesting." (P3, FG1)
	1.2 Study poster			"I spotted the poster thinking back and thought that looks interesting and then the process was much the same after that but yeah I saw the poster and that's how I got involved." (P6, FG1)
		1.2.1 Important to show financial compensation		"it's important to say you on the poster that you will be compensated for the time you spend, I don't think there's a need to put a number on it." (P5, FG1)
		1.2.2 Use of 'biting chamber' photo		"what appealed to me about the poster was that you actually showed the biting chamber because I think that was really useful because otherwise I would have thought that I'd have perhaps been in a room being bitten by flies" (P10, FG1)
	1.3 Local Link			"I actually saw the advertisement in the Local Link which is a free magazine that people in the area receive." (P16, FG2)
		1.3.1 Almost went unnoticed		"there was nothing particularly about it that, other than the fact that I knew what it was about. Had I not been interested or had ever done scientific research before I probably wouldn't have noticed it" (P15, FG2) "It looked exactly like a job advert." (P15, FG2)
		1.3.2 Unaware of article		"P2: I was interested to know if you'd linked up that there was an article and the advert. P16: No.

				P15: I don't think so. But I suppose that's fine because some people that read the whole local link would have read the article and then looked for the ad and then some people wouldn't have done and seen the ad anyway." (FG2)
		1.3.3 Perceived as 'junk mail'		"it's not that kind of popular is it as like a go to thing it does come through a lot of people's letter boxes as junk mail but yeah it will be seen as junk mail by a lot of people" (P15, FG2)
	1.4 Directly approached			"I was working in Q Block on the heating system and they said "would you like to do this?" and I said "yes" and that was it." (P7, FG1)
	1.5 Word of mouth			"somebody had told my wife and my wife told me and me and my wife was going for it. Unfortunately she wasn't successful due to health reasons but then I've been on the, I've been on it so it was all word of mouth." (P13, FG2)
	1.6 Overall suggestions for improvement	1.6.1 Using verbal methods		"some people are more easily persuaded by verbal means than written because we do live in a time of like too much information syndrome […] when everyone's presenting you with so much information I think on some level you just dismiss all of it." (P7, FG1)
		1.6.2 Emphasise end goal		"it [the study poster] didn't really sell what you wanted to do because the research it wants to help people all over the world that are affected with this fly. I think if they'd have focussed on actually people that want to save the world and help out it might have got more people in." (P14, FG2) local flyers
		1.6.3 Written content less vague / more eye-catching		"had I not been sat in front of it [study poster] was probably not as eye catching as it might have been." (P6, FG1)

				"it definitely could have been more eye catching. If you wanted more people to respond and it need to have more prominence then yeah you could have done with a bigger word, a picture you know, something, a slogan" (P15, FG2)
		1.6.4 Professional design support		"it's [i.e. study poster] the sort of thing you want like an advertising executive to design. Something that will really jump out to the lay person" (P7, FG1)
		1.6.5 Alternative methods suggested		"maybe you should do like a Facebook ad, I don't know, something on social media." (P15, FG2) "doing a little promotion video about the issue." (P15, FG2) "you could do on Minster Fm or Radio York would probably do an interview someone about it if you tell them about it." (P15, FG2) "local flyers and posters" (P13, FG2)
	1.7 Study website	1.7.1 Clear and informative		"it [the study website] was good, it was clear. I can't remember a lot about it other than the image and that it gave me the information I needed." (P3, FG1)
		1.7.2 Unaware of website		"I can't remember seeing the website, the link or anything. I don't recall…" (P16, FG2)
		1.7.3 Only used for recruitment		**"did you just go on [to the study website] at the point of wanting to take part in the project?** P5: Only when I was looking to take part. P (several): Yeah. **I: So you didn't use it as a resource beyond that?** P (several): No." (FG2)

		1.7.4 Reasons for not using website		"P6: I didn't think it was necessary. There was enough communication with Viv and everyone else to not need to use the website again. P10: Yeah we'd plenty of opportunity to ask questions to the people we were dealing with didn't we. P3: Yeah I agree." (FG1)
		1.7.5 Interested to access now aware		"I would be interested to know what's on it now. Does it have examples of other studies going on you know sort of globally Leish challenge or are there stories on it that tells you about people that are working in the countries with the victims or you know it would be interesting to know whether it, how much of a story it tells." (P15, FG2)
		1.7.6 Suggestions for improvement		"If I'd have seen it electronically I would have clicked on it. You know what I mean. I wouldn't have gone and typed it into Google." (P17, FG2)
2. Quality of participant-facing information	2.1 Well-received			"I think the [study information] sheets were completely adequate." (P15, FG2)
	2.2 More info re: extent of medical examination needed			"P10: it may well have been I didn't read it closely enough. That could be quite- P7: Yeah Viv did say to me. P10: I knew I was going to have a medical. P7: A full medical. P6: No I agree I didn't appreciate it would be quite as extensive." (FG1)
	2.3 Extensive medical - surprised but valued			"I thought it was quite nice. I know I'm healthy. I just didn't realise that was going to be as extensive as it was." (P6, FG1)

	2.4 Expressed interest in study outcomes			"I would be intrigued to see the progress of the research and where it goes and how successful it becomes…" (P14, FG2)
	2.5 Information needed for future study			"it [the future study] will take a lot more information to process in terms of I don't know what are the symptoms of the disease, what are the treatments, the potential sequels of either of those so it's a, I would think it's quite different" (P5, FG1)
3. Arrival process	3.1 Well-informed re: travel and parking			"it was very helpful the way they described how to, you know I said I'd be coming on a bicycle over Walmgate stray and they sort of told me exactly where I needed to go" (P16, FG2)
	3.2 Problem with Google Maps			"P14: I used Google maps so it took me to the other campus but then Nicky met me- P13: I did that two weeks previously." (FG2)
	3.3 Access to parking			"parking here was no hassle at all so it was very, very straightforward that for me and that made all the difference when I don't have to come onto campus and find a parking space and like you know I wouldn't have done it because I'd have just thought this is just going to be such a headache for me to do this." (P3, FG1)
	3.4 Having to wait outside if early	3.4.1 In the cold		"a couple of times I arrived early at the lab bit and I'd cycled and it wasn't very nice weather but I had to kind of stand outside and wait until the exact time to be admitted into the building." (P16, FG2) "they had problems taking my temperature because it was so cold I had to warm up. So if

				you were to sit inside you might have warmed up a bit quicker." (P13, FG2)
		3.4.2 Unaware of reception		"[you could] let people know that you can just go round the corner to the main entrance of Biology and there's a lovely big lounge area that you can sit in" (P15, FG2)
		3.4.3 Suggested doorbell		"they did say that for the next one [i.e. study] they were going to get a doorbell so you could ring in." (P13, FG2)
4. Screening process	4.1 Beneficial and accepted			"you're part of the study and that it's part and parcel of it so you get weighed and everything so you just accept it" (P16, FG2)
	4.2 Uncovered underlying health conditions			"I had the suspicion of a hernia but I was sort of putting it off until I told Dr Viv about it and he investigated and said yeah, it is a hernia and you need to get it sorted properly so yeah it was very beneficial." (P13, FG2)
	4.3 Study team were accommodating			"I know Nicky had to make quite an effort to come in earlier and I appreciated that." (P10, FG1)
	4.4 Experience of specific tests	4.4.1 Blood tests	Problems	"the first time I had blood taken they couldn't get any blood out of one arm and so they had to do the other and that arm got quite bruised and was quite sore and it was quite, there was quite a lot of discomfort. The other subsequent visits were fine and it was very painless" (P3, FG1)
			Fine and painless	"P16: I didn't have any problems with the blood tests, no. P13: No, absolutely fine." (FG2)

		4.4.2 Disliked weighing		"I didn't like being weighed." (P3, FG1)
		4.4.3 Pregnancy testing	Anxiety surrounding	"it's vanishingly unlikely I could be pregnant there's still a certain anxiety associated with having a pregnancy test to me." (P10, FG1)
			Grateful	"very grateful for the pregnancy test, thank you." (P15, FG2)
			Preference to take urine sample pots home	"for me it would have been easier to have a pot to take home." (P10, FG1)
		4.4.4 Height measurement superfluous		"it takes almost no time [to take height measurement] but it's like it's everyone's time writing it down, it's a section on the form I mean it's just a waste of resources. But then most of them make sense I mean they are taking I mean I don't know the blood test makes sense, the temperature, the weight, other stuff makes sense I think only one is a bit kind of weird is the height."
5. Experience of being bitten	5.1 A positive experience overall			"It was just a really nice experience. For me I mean, you know, I just found it all mod cons and everything was very comfortably ran because you just sit in a chair and back and yeah it was really nice, you know for my needs it was above and beyond what I needed." (P7, FG1)
	5.2 Perception of the flies	5.2.1 Smaller than anticipated		"P14: They were quite small yeah. I was expecting a bit bigger. P15: Because you couldn't really see them." (FG2)
		5.2.2 Expecting more flies / bites		"I was expecting more flies in there than just five. Not knowing what was going to be happening but I just expected more bites." (P13, FG2)

	5.3 "Unremarkable" – everything as expected			"It [the bite] was unremarkable to the point where I forgot I was taking part in a medical study." (P7, FG1)
	5.4 Physical side-effects	5.4.1 Skin reaction less than anticipated		"[The bite itself] was really minor, much less, I mean just a tiny red mark and I was expecting you know a kind of a horrible itchy lump so it was much less than I expected." (P12, FG1)
		5.4.2 Not painful		"I only got bitten properly once and I could just feel the slightest sort of prickling, nothing painful." (P10, FG1)
		5.4.3 Blistering		"It was very raised and rounded, it was like a small blister." (P13, FG1)
		5.4.4 Itchiness - needed antihistamines		"[The bite] was just itchy so the more I itched it and then it got the skin slightly torn and then just looked like an insect bite, just a scratched insect bite that I would itch which made it worse." (P15, FG2) "I did get the antihistamines on my final visit just so that I could take some when it was itchy." (P15, FG2)
		5.4.5 No medication needed		"Just didn't need to [take antihistamines]" (P13, FG2)
		5.4.6 Glad of reaction		"I was happy in a way that I did have a slight reaction that something was happening." (P13, FG2)
	5.5 Curiosity as flies began to bite			"Slightly curious, really just like ok let's do science here. Strap it on and off we go […] You've been bitten by and insect before, you're going to volunteer to be bitten by seven now, let's see what it does." (P7, FG1)

	5.6 Excitement as flies began to bite			“Really excited when they started biting.” (P10, FG1)
	5.7 Pre-bite anxiety			“I was slightly anxious that morning because I thought, I know it’s only a small area but I was thinking if they really really bite, I mean I come up quite badly with mosquito bites” (P10, FG1)
	5.8 Bite procedure	5.8.1 Longer than anticipated		“the actual bite itself was about three hours in total with the half an hour of biting and then all of the monitoring stuff which did take out the afternoon.” (P6, FG1)
		5.8.2 Suggestions for improving trial environment	Comfortable chair	“You could have had a slightly more comfy chair to sit in. […] Because I was sort of sideways to the desk and you were stretching your arm out for quite a long time.” (P10, FG1)
			Refreshments	“it would have been nice to have been able to maybe make a cup of tea or coffee in that because it was quite a long time.” (P16, FG2)
			Entertainment	“I think the option perhaps for somebody to watch a film or, you know and then just be monitored in between, might be helpful for some people.” (P10, FG1)
			Use of non-clinical environment	“[After the bite procedure] I thought you’d then get to sit in like a lounge area rather than the same clinical room, yeah. It didn’t matter at all but that’s just what I had in my head. That those three hours would be a little bit more comfortable in a way.” (P15, FG2)
		5.8.3 Well-informed		“they explained it really well as well so they explained the procedure “Oh there’s a fly bitten you and you can see it

				sucking up the blood now" (P14, FG2)
	5.9 Post-bite diary	5.9.1 Paper-diary fine		"Well I work better with paper. I'm still a dinosaur when it comes to all the sort of technology and everything so for me it was fine to have to you know didn't forget and took it with me and everything so yeah." (P16, FG2)
		5.9.2 Suggestions for future		"if you really wanted to bother like an app for you to quickly fill in your diary every day would have been amazing." (P15, FG2)
	5.10 Aftercare	5.10.1 Confident re: how to contact team		"If I'd needed to get help or something I felt quite confident that I would have been able to." (P17, FG2)
		5.10.2 Could not remember advice given		"I can't remember what the advice was." (P15, FG2)
		5.10.3 Good support network		"it seemed that everything was in place fine." (P13, FG2)
6. Overall study experience	6.1 Described as positive			"it was sort of a privilege to be a part of but like you say Viv had explained everything so well from what I say paperwork I never visited the website but anyway it was so clear there was not one point in the entire process for me was there any surprises it was exactly as sold and you know you just did it and it was fine, no surprises, no downsides, for me I mean it was a positive pleasant experience. I didn't, no apprehension at any point, there was no worry I mean I suppose I was just so well informed, due credit to them really." (P7, FG1)
	6.2 Well-informed re: entire study			"they [the team] made sure you understood especially the first ones they would constantly ask you do you have another question and kind of repeat things just to

				make sure you didn't miss anything which I think is really good." (P5, FG1)
	6.3 The study team	6.3.1 Professional		"it [involvement with the study and study team] was the most professional thing that's happened to me in a long time." (P7, FG1)
		6.3.2 Good communication		"I was expecting a phone call and I got a phone call from Viv I think it was and it was all quite seamless I felt and I think when you sign up for something you want someone to respond and you don't want to feel like you've made the effort to send an email and no one's replied for ages but it was a good follow up I think." (P3, FG1)
		6.3.3. Providing opportunities to ask questions		"we'd plenty of opportunity to ask questions to the people we were dealing with" (P10, FG1)
		6.3.4 "Warm"		"I'd applied for scientific research before but it wasn't as like, as warm as this one." (P14, FG2)
		6.3.5 Enjoyed social interaction with		"they [the team] always had lots to talk about and so it was more enjoyable rather than being with someone who's quite straight edge and quite strict and you know not really open to talk to or you know that kind of person so yeah that was quite pleasant." (P14, FG2)
		6.3.6 Good relationship with		"you build up a bit of a relationship [with the team] don't you, you get to know them, have these chats with them and it was really pleasant. They were waiting for me every time I arrived there." (P3, FG1)
	6.4 No safety concerns			**"Did you have any safety concerns at all when you**

				were part of the project? The trial. P (Several): No. **I: None at all?** P (Several): No." (FG1)
	6.5 Felt appreciated, valued, well cared for			"It was one of the nicest things about it that you felt that you were all involved in something interesting that they were genuinely excited about and that they appreciated your time and volunteering so that was really nice." (P10, FG1) "we were treated very well especially as I say when the notice about the high blood pressure and Dr Viv was like you need to get it checked throughout the visit he was quite, he seemed genuinely concerned about it which was nice, he wasn't just treating you like a number he was treating like a person and taking care of you." (P13, FG2)
	6.6 Time commitment	6.6.1 More than anticipated		"for the three hours I took a half day holiday and for this I'm trading time in lieu that I worked over Christmas so that's a commitment that I've had to make, which is fine but time commitment is an issue for most people I think." (P10, FG1)
		6.6.2 Grateful for flexibility offered		"they [the team] were prepared to be flexible with the times for you to make it as convenient as possible, the time to come and meet with them so that helps a lot too." (P5, FG1)
		6.6.3 Virtual follow-up versus in-person contact		"if it [taking bite photos] was beneficial to the study then I wouldn't mind doing it." (P13, FG2)

				"you realise that whatever was going on was perfectly normal but you know had there been some sort of reaction or whatever you would like to think that you were being seen and they would pick something up or whatever it was because if you hadn't shown the bite you might think it was perfectly normal for it do something." (P17, FG2)
	6.7 Thoughts on level of remuneration	6.7.1 A satisfactory amount, about right		it [the level of remuneration] was about right for the time commitment and for the amount of blood." (P10, FG1)
		6.7.2 Would have taken part for less		"I'd have done it for a lot less. I didn't, although I did it primarily for money I suppose I would have been perfectly happy well with sort of twenty quid…" (P10, FG1)
		6.7.3 Money a bonus, secondary		"it was secondary for me, it was very useful for me as I don't earn at the moment it's very nice to have that bit of extra cash but I actually was just really interested in the project." (P6, FG1)
		6.7.4 Money as key motivator		"it was a very interesting study but I think the main reason was I thought, ooh here's a study I can get paid for and raise some money." (P10, FG1)
		6.7.5 Importance of versus not relevant		"getting some compensation makes you feel like your time is valuable, you know I have very little free time and to do it for nothing and take a lot of time off I would be reluctant to do that […] I don't think everyone should have to do it for free, if you'd like to it's not a bad thing to get a bit of money as part of it." (P3, FG1)

				"it [the remuneration] wasn't relevant, I actually waivered my compensation so I don't. I mean I checked it the time commitment was doable as I work on campus and I have flexible hours I could work around it and ok it looks like I can do it so I went for it, I didn't really consider it." (P5, FG1)
	6.8 Reasons for taking part	6.8.1 Interest in condition/research		"I'd heard about the condition before but also I've taught, you know having taught biology for a long time and sort of talked about taking part in scientific research and I thought well you know how about me doing it myself you know so it was just an interest really" (P17,FG2)
		6.8.2 Trial meaningful and worthwhile		"It was just nice to have done something maybe a bit meaningful for a change…" (P7, FG1)
		6.8.3 Desire to help others		"it's my chance to sort of contribute to some sort of research which in the long run is going to hopefully benefit quite a few people." (P16, FG2)
	6.9 Considerations for future study			"I think it [the study] needs to be promoted more for what the end goal is rather than the actual process of it cos then I think more people like myself would be intrigued because they want to help things, they enjoy research, they enjoy helping things" (P14, FG2)

Table 7.4: Focus group table of themes

Adapted from a table produced by me, GJ and NM for (Parkash et al., 2021a).

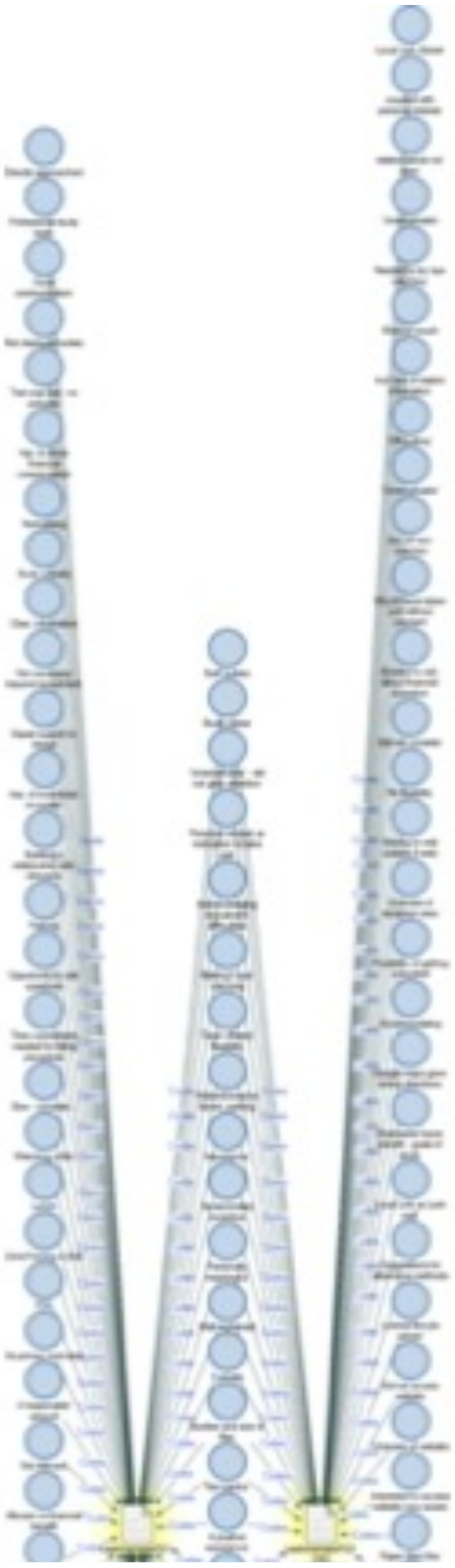
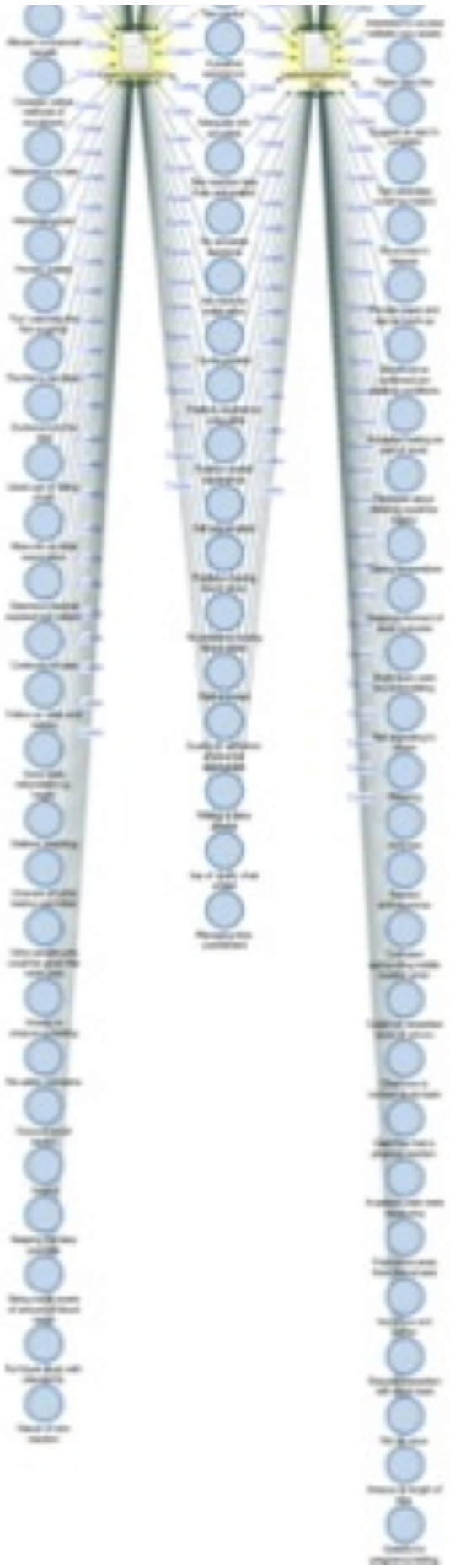


Figure 7.18: FLYBITE focus group

Coded comparison diagram



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E-Mail: vivak.parkash@york.ac.uk

A clinical study to develop a controlled human infection model using *Leishmania major*-infected sand flies – Pre-screening questionnaire

Volunteer Name _____

DOB (dd/mm/yyyy) _____

Email address _____

Contact telephone number _____

Please respond with Y / N / NK (Yes, No, Not known)

The participant:		Response
1	is aged between 18 – 50 years old	
2	is available for the duration of the study	
3	has travelled to any country outside of the EU in the last 10 years (please list dates of travel and countries below)	
4	has a history of diabetes	
5	has a history of hayfever, asthma or eczema (please list any treatment, admissions to hospital, specialist input or any other relevant details below)	
6	has a current chronic illness requiring hospital specialist input or any other significant ongoing medical condition	
7	has a history of allergies to any medicines and non-medicines (if yes please list below)	

*This refers to regions where *Leishmania major*-transmitting sand flies are endemic including (but not limited to) the Middle East, Sub-Saharan Africa, and Asia

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Any information contained within this questionnaire will not be retained and will be destroyed if participant does not proceed with the study

Figure 7.19: LEISH_Challenge pre-screening questionnaire

Department for International Development

MRC Medical Research Council

HULL YORK MEDICAL SCHOOL

UNIVERSITY of York

LEISH_Challenge Poster 1 v0.2 19-Oct-21 [EXAMPLE FOR FILE]

Would you like to help the tens of thousands of people affected by

LEISHMANIASIS

each year?

Are you 18 – 50 and in good health?

By being part of a human challenge study, you will be laying the foundations for vaccine development and potentially saving thousands of lives.

This project is funded by the Medical Research Council and Department for International Development

leishchallenge.org

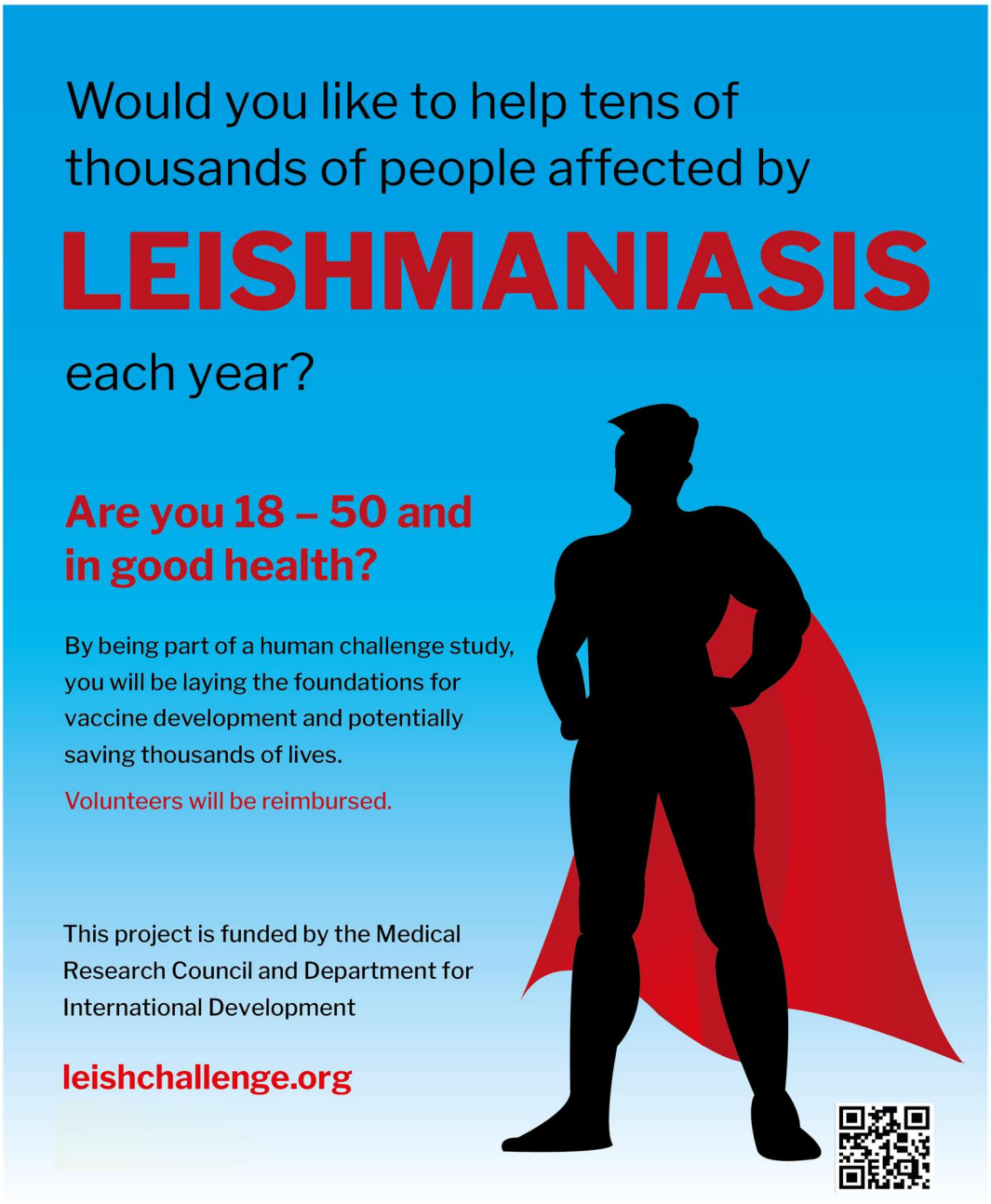
Department for International Development

MRC Medical Research Council

HULL YORK MEDICAL SCHOOL

UNIVERSITY of York

LEISH_Challenge Poster 2 v0.2 19-Oct-21 [EXAMPLE FOR FILE]



LEISH_Challenge Poster 3 v0.2 19-Oct-21 [EXAMPLE FOR FILE]

LEISH_Challenge Poster 4 v0.2 19-Oct-21 [EXAMPLE FOR FILE]

Figure 7.20: LEISH_Challenge advertisement posters

LEISH_Challenge:

A *Leishmania major* human challenge study

Participant Information Leaflet

For more information contact the project team at York:
email: vivak.parkash@york.ac.uk phone: 01904 328621

Background

The University of York would like to invite you to take part in a research study, "A clinical study to develop a controlled human infection model using *Leishmania major*-infected sand flies".

Our research relates to an infection called leishmaniasis which mainly occurs in tropical countries. It affects millions of people and causes around 20,000 deaths across the world every year. There are different types of leishmaniasis around the world and some can be very serious. They affect the skin (cutaneous leishmaniasis) or the internal organs of the body (visceral leishmaniasis). Some of the milder forms will produce skin problems which will be localised, whilst other forms of leishmaniasis will cause widespread skin changes. The skin lesions (an abnormal change in the skin) of cutaneous leishmaniasis can be disfiguring if left untreated.

Our group is designing a type of research study, known as a controlled human infection model (CHIM), whereby human participants are deliberately exposed to infections. This approach of exposing humans to infectious diseases has been used for hundreds of years as a method for better understanding how diseases progress and for the evaluation of vaccines and treatments. Vaccine development for neglected tropical diseases has much to gain by the use of CHIMs, and this approach has already been used in developing vaccines for cholera, malaria, influenza and dengue fever. No vaccines are currently approved for leishmaniasis, a disease affecting millions each year. However, new candidate vaccines have emerged over the last few years, emphasising the need for a CHIM for leishmaniasis.

What is the purpose of the study?

Our objective is the development of a controlled human infection model of *Leishmania major* using sand fly transmission which is effective and safe. Leishmaniasis is caused by the Leishmania parasite and is transmitted by sand flies. The parasite is tiny and not visible to the naked eye, whereas the sand fly is visible but small and inconspicuous.

It has been shown that sand fly saliva is crucial for optimal infection. In addition, studies using mice injected with the *Leishmania* parasite have shown that using a needle to inoculate the parasite, rather than using sand flies, may be unreliable when testing vaccines. For these reasons, this study will be using sand flies to infect human participants in a controlled human infection model with Leishmaniasis.

In late 2019, we conducted an initial successful study using uninfected (disease-free) sand flies. Information from the original study will inform the way we work this time using **infected** sand flies.

The aim is to develop a model that we can use in the future to assess vaccines against Leishmaniasis. We will **not** be testing a vaccine in this study.

We will be using a species known as *Leishmania major* which does not cause severe disease but causes a localised lesion in the skin at the site of a sand fly bite. This can appear similar in appearance to a spot. This lesion can be treated with several known treatments for leishmaniasis, and in this study will be using a small biopsy to remove the lesion. We believe that usually no further treatment will be necessary, but if it is we may use a freezing treatment (cryotherapy). We will also ask your permission for an optional small biopsy of healthy skin from the opposite arm. This will help us to compare how the skin reacts to the *Leishmania* parasite.

Bear in mind that in addition to the clinical interventions and observations, we will also ask you to take part in a focus group with other participants which will be audio recorded with your consent.

Before agreeing to take part in this research, please read this information sheet carefully and let us know if anything is unclear or you would like further information.

Do I need to do anything beforehand?

Please take any regular medications ensuring that the study team are aware of these. If you have been started on any new medications it is extremely important to tell the study team as soon as possible before you attend. You may eat and drink as normal but a light diet is suggested.

Please wear loose clothes or a T-shirt for example, to ensure that the area of skin on your arms are easy to access and exposed. It is advised to bring a book or some reading material in case you have to wait for any period of time.

What happens if there is a problem?

If you feel at any stage that there has been a problem with your participation, you can discuss this matter, in confidence, with Professor Charles Lacey, the Chief Investigator, on: tel 01904 328879 or email: charles.lacey@hyms.ac.uk

Why have I been asked to take part?

We are advertising for volunteers to take part in this study. Volunteers must be men and women aged between 18 and 50 years old. Enrolled female participants must **not** be pregnant or breastfeeding and must be using an effective form of contraception. Volunteers must be healthy and **not** be at risk of serious infections and must **not** have any chronic skin conditions. Volunteers should **not** have travelled in the last 30 days to any area where leishmaniasis is present or for 30 consecutive days at any time in the past. There should be **no** history of significant reaction to insect bites. A full review of any possible reasons that could prevent you from taking part will be performed by the medical team if you express an interest in the study.

Where do the sand flies come from?

We are working with scientists in the Czech Republic who are insect experts (entomologists). They have been rearing sand flies for several years and are experts in developing sand flies for use in research. We have been working closely with these scientists who will provide us with the right kind of sand fly for our study. There will be up to 2 different types of sand flies used, both of which can transmit *Leishmania.*

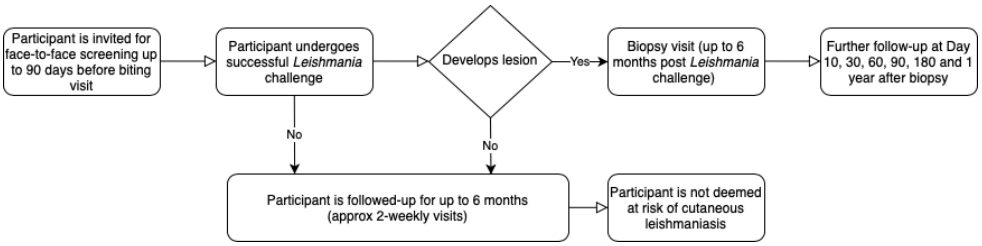
Where does the Leishmania parasite come from?

Although there are several laboratories and parasite banks around the world that have parasites available for use in experiments, we wanted to know the precise origin of the parasites that we use to be able to prove their safety and effectiveness. Therefore, we have obtained fresh parasites from healthy individuals in Israel who developed a *Leishmania major* lesion which was not serious, healed well and from which they recovered fully. This parasite has then been tested by laboratories in Israel, Czech Republic, Germany and York to check some of the safety aspects, and to make sure it will be effective in our proposed model

What will happen if I agree to take part?

This study is designed to test the safety and effectiveness of the *Leishmania* human challenge protocol in healthy volunteers.

The number of study visits will depend on when a lesion develops as detailed in the diagram below. All visits will take place at Translational Research Facility, Q Block, Department of Biology, University of York, York, YO10 5DD.



- There are 3 phases, as shown above
    - Phase 1 - The screening visit for the study
    - Phase 2 - The leishmania challenge, and then the follow up until a leishmania lesion (spot/lump) develops (or otherwise, if no leishmania lesion develops)
    - Phase 3 - Then if the leishmania lesion (spot/lump) appears, the treatment and follow up phase
- In Phase 1, we will first ask you some screening questions by email and/or telephone contact, before inviting you for the formal screening visit:
    - **Study visit 1**: Screening including blood tests will be performed to assess suitability. This can take place up to 3 months before the main study. This will last an hour.
- Phase 2:
    - **Study visit 2**: The 2nd visit will be the leishmania challenge itself. Using a wrist watch-like biting chamber, 5 infected sand flies will be placed against your skin for 30 minutes. Photography and videography of the bite site will be performed with your permission. You will be observed for a period of time before being allowed to go home. A doctor and a nurse, or two doctors, and an insect specialist (entomologist) and other senior study investigators will be present throughout. This session will last around 3 hours. You will be asked if you agree to provide an optional video-recorded account of your experience.
    - **Study visits 3-5:** Follow-up visits will take place at approximately 4 days, 14 days and 28 days after the biting visit. These will be 30-minute visits for examination of the bite site and blood tests on days 14 & 28. It is expected that a cutaneous leishmaniasis lesion will develop at some point from 28 days onwards.
- After visit 3, participants will have ongoing follow-up until they develop a cutaneous leishmaniasis lesion. **Study visits 4-11**; these will take place at the following timepoints following sand fly biting: 2 weeks, 4 weeks, 6 weeks, 8 weeks, 10 weeks, 14 weeks, 18 weeks and 22 weeks. Study visits 6, 8, and 10 are planned to be 'virtual visits' by video calling for examination of the bite site. Study visits 5, 7, 9, and 11 will be 30-minute 'actual visits' for blood tests and examination of the bite site.
- If by the time of **visit 11** (22 weeks) no cutaneous leishmaniasis lesion has developed, then we will presume that no leishmania has been transmitted. You will be given a leaflet with details of the study team in case any issues or problems develop later. Your GP will also be informed at this stage, and no more study visits will be scheduled.
- Once a cutaneous leishmaniasis lesion is present at any study visit that is greater than or equal to 3mm in diameter, you will be followed-up in a **Biopsy visit**. This will involve a small biopsy to remove the lesion (see description in 'What are the potential risks of taking part?'). The biopsy visit will reset your schedule of follow-up visits as follows:-
- Following the biopsy visit, a further visit will take place at 10 days to check healing of the biopsy site.
- Further follow-up will then take place at 30, 60, 90, 180 days and the last study visit at 1 year following biopsy treatment. It is anticipated that some of those visits can be 'virtual' using video calling.
- We would also like volunteers to take part in a focus group. This will take place after the last study visit and it is anticipated that this will last between 2-3 hours and involve the participants that have taken part in the study. The focus group will be led by Prof Georgina Jones, a health psychologist, and provide the opportunity for you to share your experiences of participating in the study and any concerns you had, and help us to shape the design of the next stage of the research. The Focus Group will take place at the University of York, and

non-identifiable information will be recorded and will contribute to future publication and research.

Blood will be taken at Study visits 1, 4, 5, 7, 9, & 11. Blood will also be taken on the day of the biopsy, and also on days 30, 90 and 360 (last study visit) following biopsy. Approximately 50 ml of blood will be taken each time. There is a small risk of bruising following the blood tests. We will ask your permission to contact your GP to confirm your eligibility prior to starting the study.

We also want to try and measure if the research study causes you any stress or upset, and whether the bite site or cutaneous leishmania lesion causes you any or much discomfort or difficulties. To do this we will ask you to complete two brief questionnaires, known as GAD-7 & DLQI, throughout the study. We estimate these take 5-10 minutes total to complete and will be performed at Study Visits 1, 4, 5, 7, 9, & 11 Following the biopsy, these questionnaires will be recorded at every further visit.

What is a biopsy?

A biopsy of the skin is a very simple procedure performed in the outpatient setting which takes 10 to 15 minutes. A small sample of skin is removed and then examined by the study team. There are two possible ways in which this can be performed either an 'excision' biopsy in which a lesion is fully removed or an 'incision' in which a small sample of a skin is removed. The latter is often done with an instrument called a 'punch' device.

In both procedures the skin is cleaned to reduce risk of infection and then the skin is 'anesthetised' (numbing agent used to prevent discomfort during the procedure). A local anaesthetic is injected just underneath the skin to numb the area. This can cause a stinging sensation but will quickly subside and the area will become numb.

In an 'excision' biopsy, the area to be removed is drawn out on to the skin. In this study this is likely to be up to 2cm by up to 1cm in size (approximately the size of a jelly bean sweet). The skin is then removed with a small knife called a scalpel. 3-4 small stitches will then be used to bring the sides of the excision biopsy together to help with healing and a dressing put over this.

In a 'punch' biopsy a small pencil like device which looks like a miniature apple corer is used to remove a small sample of the skin. This is a much smaller version of a hole-punch device. 2-3 small stitches will then be used to bring the sides of the skin together to help with healing a dressing put over this.

Your doctor will discuss which of the above techniques will be used. You will be awake throughout and will be able to talk to the doctor throughout the procedure.

What is the purpose of the biopsy?

The excision biopsy will be used a treatment for cutaneous leishmaniasis, to stop the lesion growing further. This method has been used in other centres for this purpose. We will also be analysing this skin sample in the laboratory to look for evidence of Leishmania parasite and to perform other tests which will help in our study and future studies against leishmaniasis.

If you have agreed we may also perform a biopsy of healthy skin on the opposite arm to the *Leishmania* lesion. This is so we can compare what happens to an individual's healthy skin versus infected skin following a sand fly bite. We will use the punch biopsy technique to do this to ensure that we take the smallest possible area of skin.

Who is organising and funding this study?

Funding has been provided by the UK Medical Research Council (Ref: MR/R014973/1)

What are the potential risks of taking part?

Volunteers may experience a 'pin-prick' sensation when bitten by sand flies. Common effects following sand fly bites (as with all insect bites) include some swelling, pain and redness at the bite site. This may take up to 48 hours to develop. Just as with any insect bite there is the potential for a skin infection which may require antibiotic treatment.

The species of sand fly that we are using has not been associated with any serious reaction in the past. Although hundreds of thousands of people are bitten by sand flies every day without any serious effects, we want to absolutely ensure your safety. For this reason a health check and blood tests taken prior to the study will help to identify volunteers who are more likely to suffer adverse effects and these individuals will be excluded from participating further (this includes a blood test to look specifically at sand fly bite reaction as well as other reactions).

To date, there have been no reported cases of anaphylactic (life-threatening) reactions to sand fly bites in the whole medical literature. However, there might still be a small theoretical risk of serious reaction to sand fly bite, just as with any insect bite. When a serious reaction occurs, although rare it usually presents with breathing problems and widespread skin rash. Within the very small number of people who develop a serious reaction, a small number of these may be at risk of death. We have appropriate treatment and a medical team on-site at all times in the very unlikely event that a serious reaction occurs.

We can tell quite accurately if sand fly biting has occurred, and in our previous study we were 100% successful. In the unlikely event that a participant does not develop any kind of bite or spot in the first four weeks, it is very likely that the sand flies did not transmit any parasites to the participant. If this occurs, the participant will only have regular follow-up till 28 days and then a final check at around 6 months to ensure that all is well.

We expect participants will develop a cutaneous leishmaniasis lesion at the sand fly biting site. This lesion can appear similar to a spot and we expect it to develop during the first 5 months after biting. There are no recorded cases of significant ill-effect from this in healthy individuals. The lesion will be allowed to grow up to 3mm in diameter (about one eighth of an inch). The sand flies are reared free from harmful infections. The sand flies will be infected with the *Leishmania major* parasite at the University of York. If left to grow without treatment, the lesion can develop into a sore known as an 'ulcer', although a number of individuals with this lesion have gone on to 'self-heal' without any treatment.

It is possible that after the biopsy there may still be some parasites left in the body near the site of infection. Normally these help to keep the immune system stimulated and provide protection against reinfection. This might be beneficial if the participant travels to a leishmaniasis endemic region and is exposed naturally. However, the presence of this small number of parasites makes it possible that immunosuppression (e.g. through an organ transplant, HIV or drugs that affect the immune system) could cause reactivation of the lesion. Although this can happen in other forms of leishmaniasis, we are unable to find any descriptions of this occurring with *Leishmania major* after successful treatment.

Just like with any medical procedure there are some risks involved with the biopsy. These are rare but are important to know about and include:
Pain
Infection
Bleeding
Incomplete excision of the lesion. This might mean a repeat biopsy is needed.
Bruising
Numbness at the scar site
Small scar (similar to a chicken pox scar)
Very rarely:-
A small bump might form at the scar site but if this occurs, it is likely to settle over time.
Wound breakdown and ulceration

As treatment, we intend to perform an 'excision biopsy' once a lesion develops. This will occur when it gets to about 3mm in diameter. This is a simple outpatient procedure which takes about 15 minutes. This procedure is performed in a sterile fashion, using local anaesthetic. The local anaesthetic can feel like a sharp scratch which will last a few seconds. Once the area is numb and no pain is felt, a small ellipse centred on the spot, approximately 18 mm x 7 mm (¾ x ¼ inch), will be removed using a scalpel. The long axis is oriented along the natural lines of the skin to get the best cosmetic result. A very small amount of blood may be visible immediately following the procedure. 2-3 small stitches are used at the biopsy site. For 2 days, contact with water just at this site should be avoided.

Some individuals may develop a very small scar at the site of the biopsy although this is likely to be minimal. Some individuals may notice a small bump at the site of the lesion. The stiches will be removed after 10 days.

If you feel unwell at any point during or after the study you will be supported by the research team and given any necessary medical attention. This will include a support line for advice if needed and admission to hospital, although this is thought to be very unlikely.

What precautions will you take to reduce the risk of COVID-19?

The study team involves doctors who are used to dealing with COVID-19 and wearing PPE (personal protective equipment). The team will adhere to all current UK Government guidelines. In addition all research will be approved by NHS and University research committees with a risk assessment carried out to mitigate any risks.

The study team will adhere to up-to-date clinical practice, and use of any necessary PPE. A dedicated entrance is used to enter the facility and there will be no contact with non-study team members. The study team is small and will likely comprise of 3 members all wearing PPE if deemed necessary by NHS practices. Good hand-hygiene practices will be employed throughout as is common practice in our clinical facility, and our facilities are cleaned regularly. Participants will be asked to wear a mask where necessary.

If you develop a fever, you must self-isolate in accordance with current government guidelines and it is feasible that some study visits can take place via video calling/phone. It must be noted that you may develop a fever following sand fly biting, although the risk of this is deemed to be very low and was not observed in our initial study.

Participants will be asked to undertake regular testing for SARS-CoV-2, if such facilities exist and are easily accessible (e.g. lateral flow testing), although this will not be mandatory.

Compensation for your involvement

Participants will be compensated for their time and for the inconvenience at approximately these rates:
Screening visit: £20
Leishmania challenge: visit £500
Follow-up visits: £40 per visit
Per completed participant-submitted photograph & diary card event: £5 (this will take place up to a maximum of once to twice weekly only in accordance with study requirements)
Biopsy visit: £500
Final visit: £100
Focus Group visit: £100
(Total up to approximately £2500 depending on involvement.)

What are the potential benefits of taking part?

The information from this study will help towards further understanding of sand fly bites and transmission of *Leishmania* parasite. This will contribute to developing a model for testing vaccines using infected sand fly bites. Ultimately this research will help in the effort to beat leishmaniasis.

You will also undergo health and wellbeing screening by the medical doctors who are involved in the study. This will involve a history, physical examination and blood tests. If there are any abnormal or unexpected findings from these screenings, this will be discussed with you by the study team and you will be referred to an appropriate medical speciality or your GP within the NHS.

Exposure to sand fly resulting in a cutaneous leishmaniasis lesion, may for some individuals protect against future leishmaniasis infection after further exposure e.g. if a participant travels to an area where leishmaniasis is present. This is a potential benefit but study investigators will be unable to quantify this.

Do I have to take part in the study?

No, participation is optional. If you do decide to take part, you will be given a copy of this information sheet for your records and will be asked to complete a participant consent form. If you change your mind at any point during the study (including during the focus group), you will be able to withdraw your participation without having to provide a reason. However given the nature of the study, and the need for ongoing follow-up, this will be discouraged after the leishmania challenge visit has taken place.

Any screening data taken prior to commencing the study will be destroyed if the participant is deemed not to be eligible to enter. Otherwise any samples and information obtained up until the point of withdrawal will be retained.

What will happen to my samples?

The blood samples will be tested at the Translational Research Facility, HYMS / Department of Biology, University of York and York Hospitals NHS Foundation Trust. This is where they will be studied by the research team. Samples will be tested in secure laboratory facilities accessible only by authorised research staff. Samples will be labelled with a unique ID number and the date only but no personal details, so they cannot be identifiable as having been donated by you. The samples will only be used in research projects that have been independently reviewed and approved by the

Department of Biology Ethics Committee. The samples will be stored for 12 months and may be used for additional future research with your permission. After this period, the samples will be destroyed.

What happens if there is a problem?

If you feel at any stage that there has been a problem with your participation, you can discuss this matter, in confidence, with Professor Charles Lacey, the Chief Investigator, on: tel 01904 328879 or email: charles.lacey@hyms.ac.uk

The University of York holds Public Liability ("negligent harm") and Clinical Trial ("non-negligent harm") insurance policies which apply to this study. If you can demonstrate that you experienced serious and enduring harm as a result of your participation in this study, you may be eligible to claim compensation without having to prove that University of York is at fault. If the injury was unrelated to the study, University of York will not be required to compensate you in this way. Your legal rights to claim compensation for injury where you can prove negligence are not affected. Please contact the Chief Investigator if you would like further information about the insurance arrangements which apply to the trial.

On what basis will you process my data (i.e. personal information)?

Under the General Data Protection Regulation (GDPR), the University has to identify a legal basis for processing personal data and, where appropriate, an additional condition for processing special category data. (Please see the additional document on data protection and personal information)

What happens next?

If you are interested in this study or if you have any questions, please contact one of the study team members (details given on the front of this leaflet). One of our study team will talk to you about what is involved and ask questions about your suitability for this study. The next step will then be to invite you to a screening assessment at our facilities at the University of York.

This project: "Development of a human challenge model of Leishmania major infection as a tool for assessing vaccines against leishmaniasis" is funded by the Medical Research Council and Department for International Development (ref: MR/R014973/1)

MRC Medical Research Council

Department for International Development

On what basis will you process my data (i.e. personal information)?

Under the General Data Protection Regulation (GDPR), the University has to identify a legal basis for processing personal data and, where appropriate, an additional condition for processing special category data.

In line with our charter which states that we advance learning and knowledge by teaching and research, the University processes personal data for research purposes under Article 6 (1) (e) of the GDPR:

Processing is necessary for the performance of a task carried out in the public interest

Special category data is processed under Article 9 (2) (j):

Processing is necessary for archiving purposes in the public interest, or scientific and historical research purposes or statistical purposes

Research will only be undertaken where ethical approval has been obtained, where there is a clear public interest and where appropriate safeguards have been put in place to protect data.

In line with ethical expectations and in order to comply with common law duty of confidentiality, we will seek your consent to participate where appropriate. This consent will not, however, be our legal basis for processing your data under the GDPR.

How will you use my data?

Data will be processed for the purposes outlined in this information sheet.

Will you share my data with 3rd parties?

No. Data will be accessible to the project team only.

Anonymised data may be reused by the research team or other third parties for secondary research purposes.

How will you keep my data secure?

The University will put in place appropriate technical and organisational measures to protect your personal data and/or special category data. For the purposes of this project we will store data on dedicated servers and / or on the University of York central data store, which provided secure long term storage for data, including daily backups, according to the University of York Research Data Management Policy.

A study master file will be created to include the study protocol, original signed consent forms and ethics/governance documentation. It will also include a list of study group participants, their email address and their telephone number. The study master file will be stored at Department of Biology in a secure, fire and rodent-proof cabinet that is only accessible to authorised members of staff.

Information will be treated confidentiality and shared on a need-to-know basis only. The University is committed to the principle of data protection by design and default and will collect the minimum amount of data necessary for the project. In addition, we will anonymise or pseudonymise data wherever possible.

Will you transfer my data internationally?

No. Data will be held within the European Economic Area in full compliance with data protection legislation.

Will I be identified in any research outputs?

No.

How long will you keep my data?

Data will be retained in line with legal requirements or where there is a business need. Retention timeframes will be determined in line with the University's Records Retention Schedule.

What rights do I have in relation to my data?

Under the GDPR, you have a general right of access to your data, a right to rectification, erasure, restriction, objection or portability. You also have a right to withdrawal. Please note, not all rights apply where data is processed purely for research purposes. For further information see, https://www.york.ac.uk/records-management/generaldataprotectionregulation/individualsrights/

Questions or concerns

If you have any questions about this participant information sheet or concerns about how your data is being processed, please contact the study team in the first instance. If you are still dissatisfied, please contact the University's Acting Data Protection Officer at dataprotection@york.ac.uk.

Right to complain

If you are unhappy with the way in which the University has handled your personal data, you have a right to complain to the Information Commissioner's Office. For information on reporting a concern to the Information Commissioner's Office, see www.ico.org.uk/concerns.

This project: "Development of a human challenge model of Leishmania major infection as a tool for assessing vaccines against leishmaniasis" is funded by the Medical Research Council and Department for International Development (ref: MR/R014973/1)

MRC Medical Research Council

Department for International Development

Figure 7.21: LEISH_Challenge participant information leaflet

Translational Research Facility (Q Block)
Hull York Medical School
Department of Biology
University of York
York, YO10 5DD, UK

Direct Telephone: +44 (0) 1904 328934
Direct Facsimile: +44 (0) 1904 328844
E-Mail: vivak.parkash@york.ac.uk

Participant consent form: A clinical study to develop a controlled human infection model using *Leishmania major*-infected sand flies
Participant number:

The version of the **Participant Information sheet** and **GDPR form** that I have seen is:	**Version**	**Dated**

	I acknowledge by my initials that:-	**Please initial box to agree**
1.	I confirm that I have read and understood the Information Sheet and GDPR form for the above study, and have had the opportunity to ask questions. I agree to take part in this study and to abide by the restrictions set out in the Information sheet. In doing so I agree to exposure to *Leishmania*-infected sand flies with the intention of developing a cutaneous leishmaniasis lesion.	
2.	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.	
3.	I understand that if I withdraw following sand fly biting I understand that I may be at risk of complications of uncontrolled *Leishmania* infection if I do not seek appropriate advice.	
4.	I understand that sections of my study documents may be looked at by responsible individuals from the University of York or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my study documents and/or medical records. This may include medical records.	
5.	I agree to be entered onto a national database (TOPS) as a measure to prevent over-volunteering and to have photograph taken for identification purposes.	
6.	I agree to allow anonymised photography and videography during the biting study and follow-up appointments, which may be used in publication.	
7.	I understand that I will take part in a focus group, which will be audio recorded and analysed, but only identified in that by my study number if I wish.	
8.	I agree that my GP (or GP representative) can be contacted for information that may affect my participation in the trial or general health, and will be informed if I decide to take part and when my involvement in the study is complete.	
9.	I agree to a biopsy of areas which develop a cutaneous leishmaniasis lesion and understand that this biopsy is used as part of treatment.	

10.	I understand that samples of my tissue, including blood samples, will be taken for analysis. I agree to these samples being stored for the purpose of this study. (This will include testing for HIV and Hepatitis B & C).	
11.	I agree that the stored samples from my participation may be retained and used for additional related future research, provided that research is given additional approval from a Research Ethics Committee.	
12.	I understand that I may withdraw consent for my samples and data to be retained and included in reports/ statistical analyses as long as sand fly exposure has **NOT** occurred. However I understand that any blood test data recorded at York Teaching Hospitals NHS Foundation Trust will still be retained in an anonymous fashion and is not suitable for destruction but will not be linked to my NHS records.	
13.	I agree not to travel to any areas endemic for *Leishmania*-transmitting sand flies for the duration of this study (including (but not limited to) the Middle East, North & Sub-Saharan Africa, and Asia). A full list of countries is available upon request.	
14.	I agree not to take pictures or video of study proceedings without the express permission of the study investigators.	
15.	**Female participants** I certify that I am **NOT** currently pregnant or breastfeeding and have not given birth within the last 3 months, and I agree to undergo pregnancy testing as appropriate.	
16.	**Female participants** I confirm that I am currently using an effective form of contraception, and I agree to continue to do so (up to 3 months post any biopsy), unless explicitly agreed with the study investigators.	
	The below statements are optional features of the study that may help investigators in additional research	
17.	**OPTIONAL:** I agree that my details may be stored in order for the study team to contact me about future research studies that may be of interest.	
18.	**OPTIONAL:** I agree to a recording featuring my voice and/or facial appearance which may be recorded during and/or after sand fly biting to record a testimonial account of the study. This may be used in further research and/or promotional material.	

Name of volunteer	Date	Signature
_____	_____	_____
Chief Investigator or person designated by Chief Investigator	Date	Signature
_____	_____	_____

Figure 7.22: LEISH_Challenge participant consent form

LEISH_Challenge Clinical Study Team
Translational Research Facility
Q Block
Hull York Medical School
Department of Biology
University of York
Wentworth Way
York, YO10 5DD, UK
Direct Telephone: +44 (0) 1904 328934
Direct Facsimile: +44 (0) 1904 328844
E-Mail: vivak.parkash@nhs.net

[Insert GP address]

[Insert Date]

Dear Dr,

RE: A clinical study to develop a controlled human infection model using *Leishmania major*-infected sand flies

We are writing to you about [insert Volunteer Name (DOB dd/mm/yyyy)] who we understand is your patient and who has consented to be entered into the above study.

Please find enclosed a volunteer information sheet, which includes the eligibility criteria for this study.

In order for us to proceed with enrolling your patient into this study we simply require **a copy of the past medical history which can be emailed to the above NHS email address**. This can be carried out by one of your administrative staff and there is no paperwork for the GP to fill or sign.

We do however ask you to read the attached information and to get in to contact with the study team if after reading the enclosed information you feel that there is any reason that the participant should not take part in this study. We will await your response within 3 weeks of receipt of this letter and after that point presume no significant issues will preclude this patient from taking part in this study.

We also enclose a copy of the signed consent form which indicates that the volunteer has provided consent for us to contact you to request this information.
Upon receipt of the past medical history we will send you an honorarium of £20.
We will keep you up to date with your patient's progress but if you have any concerns or questions in the meantime please do not hesitate to contact the research team.

Yours sincerely,

Prof Charles JN Lacey
Chief Investigator

Figure 7.23: LEISH_Challenge participant GP letter

LEISH_Challenge study

A clinical study to develop a sand fly biting protocol using *Leishmania major*-infected blood-fed sand flies

Emergency contact number: 07759 775128

Contact your study doctor in the event of any emergency or hospital admission:

For any urgent medical attention please phone 111 (or 999 if life-threatening) or attend your nearest Accident & Emergency Department.

Email: leishchallenge-project@york.ac.uk
Web: Leishchallenge.org

Participant study ID: ……

For the attention of healthcare professionals

This participant is engaged in a clinical study investigating the bite of a *Leishmania major*-infected sand fly on humans. The risks are similar to those from other insect bites and very unlikely to cause serious harm. For further information discuss with the study clinicians on 07759 775128 or email: leishchallenge-project@york.ac.uk

LEISH_Challenge contact card v0.1 (If found, please return to Dept. of Biology, University of York)

Figure 7.24: LEISH_Challenge participant emergency contact card

LEISH_Challenge study diary

This is a diary card for participants taking part in the LEISH_Challenge study.
It is intended to be quick and should not take more than a few minutes to complete.

It should be completed every day for the first 21 days of the study, and then ideally weekly.
Please feel free to add extra entries if you wish (e.g if you develop new symptoms).

For any urgent queries please call: 07759 775128

*Required

Please confirm your name or study ID *

Your answer

(Optional) The date of your answer is automatically recorded. If you are referring to a different date please state below

Date

dd/mm/yyyy

Visual analogue score (for reference with regards to below questions)

AT THE BITE SITE: Please record your symptoms on a scale 0 (no symptom) to 10 (worst imaginable symptom) *

	0 (no symptom)	1	2	3	4	5	6	7
Itch	○	○	○	○	○	○	○	○
Pain/discomfort	○	○	○	○	○	○	○	○
Redness	○	○	○	○	○	○	○	○
Swelling	○	○	○	○	○	○	○	○

GENERAL SYMPTOMS: Please record your symptoms on a scale 0 (no symptom) to 10 (worst imaginable symptom) *

	0 (no symptom)	1	2	3	4	5	6	7	8
Generally feeling unwell (malaise)	○	○	○	○	○	○	○	○	○
Muscle pains	○	○	○	○	○	○	○	○	○
Fever	○	○	○	○	○	○	○	○	○
Sickness	○	○	○	○	○	○	○	○	○

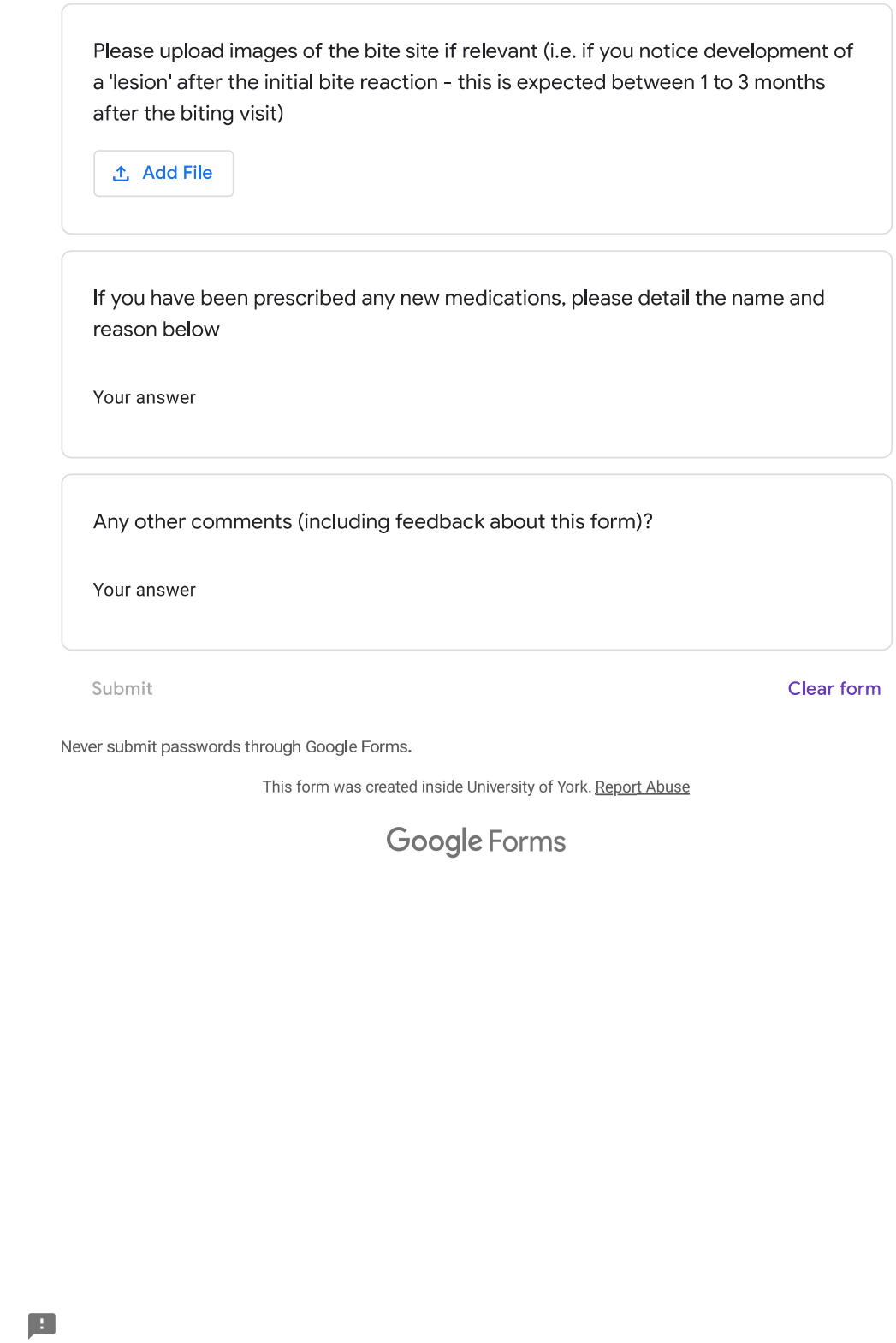


Figure 7.25: LEISH_Challenge electronic diary card screenshot

Translational Research Facility (Q Block)
Hull York Medical School
Department of Biology
University of York
York, YO10 5DD, UK

Direct Telephone: +44 (0) 1904 328934
Direct Facsimile: +44 (0) 1904 328844
E-Mail: vivak.parkash@york.ac.uk

Participant consent form: **LEISH_Challenge**
BIOPSY CONSENT FORM

Participant number:

Procedure(s) to be undertaken:

1. Excision of leishmania lesion Y/N
2. Biopsy of normal skin Y/N

Healthcare professional responsible for your care: _____

Healthcare professional statement:
I confirm I have sufficient knowledge of this procedure and have explained the procedure to the participant.

The intended benefits are: Investigation and treatment of suspected cutaneous leishmaniasis lesion and optional investigation of healthy skin

Risks involved:

- Pain
- Infection
- Bleeding
- Incomplete excision of the lesion. This might mean a repeat biopsy is needed.
- Bruising
- Numbness at the scar site
- Small scar (similar to a chicken pox scar)
- Very rarely:-
- A small bump might form at the scar site but if this occurs, it is likely to settle over time.
- Wound breakdown and ulceration
- Other (please specify)_____

The version of the **Participant Information sheet** that explains the above procedure is:	**Version**	**Dated**

	I acknowledge by my initials that:-	**Please initial box to agree**
1.	I confirm that I have read and understood the Information Sheet for the above procedure, and have had the opportunity to ask questions.	
2.	I understand the procedure will involve a local anaesthetic.	
3.	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.	
4.	I understand that if I withdraw following sand fly biting I may be at risk of complications of uncontrolled *Leishmania* infection if I do not seek appropriate advice	
5.	I give permission that my GP may be notified of health issues that may be identified through my participation if the study doctor considers this appropriate.	
6.	I agree to a biopsy of areas which develop a cutaneous leishmaniasis lesion and understand that this biopsy is used as part of treatment	
7.	I understand that samples of my tissue, will be taken for analysis. I agree to these samples being stored for the purpose of this study.	
8.	I agree that the stored samples from my participation may be retained and used for additional related future research, provided that research is given additional approval from a Research Ethics Committee.	
9.	I understand that once biopsy has taken place my samples and data will be retained and included in reports/ statistical analyses, (unless otherwise agreed in conjunction with the Chief Investigator)	
10.	I have not been notified that I am at risk of Creutzfeldt Jakob Disease (CJD) or variant vCJD	
	The below is an optional feature of the study that will help the study investigators with their research but is not mandatory	
11.	I agree to an OPTIONAL biopsy of healthy skin	

Name of volunteer	Date	Signature	
_____	_____	_____	
Chief Investigator or person designated by Chief Investigator	Date	Signature	Job description
_____	_____	_____	_____

LEISH_Challenge Biopsy consent v0.4.docx

Figure 7.26: LEISH_Challenge biopsy consent form

LEISH_Challenge:

A *Leishmania major* human challenge study

After your skin biopsy - Participant Information Leaflet

For more information contact the project team at York:
email: vivak.parkash@york.ac.uk phone: 01904 328621
Emergency contact number: 07759 775128

Background

We thank you for your participation in our *Leishmania major* human challenge study. This leaflet explains aftercare instructions following your excision biopsy procedure.

What is the purpose of the biopsy?

The excision biopsy will be used as a treatment for cutaneous leishmaniasis, to stop the lesion growing further. This method has been used in other centres for this purpose. We will also be analysing this skin sample in the laboratory to look for evidence of Leishmania parasite and to perform other tests which will help in our study and future studies against leishmaniasis.

Will I have any complications?

Just like with any medical procedure there are some potential complications. These are rare but are important to know about and include:

Pain – After the local anaesthetic wears off you may experience some mild pain. You can take simple painkillers such as paracetamol for this (ensure that you take these according to the packet or your doctor).

Infection – Your wound is expected to look red after the biopsy. If the area becomes very painful, hot or swollen, contact the study team.

Bleeding – It is unlikely that your wound will bleed after the biopsy. If you do notice any bleeding, apply firm pressure for 15-20 minutes. If bleeding is persistent or uncontrolled, contact the study team or seek medical attention.

Incomplete excision of the lesion – We think this scenario is unlikely but if this does occur, a repeat biopsy might be recommended.

Bruising – If you notice swelling, or bruising, you can use a cold compress to help alleviate this.

Numbness at the scar site – This usually settles over a few days to weeks when present.

Small scar – This may be similar to a chicken pox scar.
Very rarely:

A small bump might form at the scar site - If this occurs, it is likely to settle over time.

Wound breakdown and ulceration – If this is noted, then you may need further medical attention. If this occurs please contact the study team.

How should I look after the biopsy site?

A dressing will be applied over the area(s). The biopsy site should be kept clean. The site should not be submerged in water (i.e. no swimming, hot tubs, baths etc) for 48 hours. You may remove the dressing carefully yourself at this stage and bathe the area gently but should avoid soaking the wound for a long period of time until the stitches are removed. You can apply Vaseline from a clean pot to the wound twice a day after removal of the dressing.

It is important not to stretch the wound during the first 4 weeks as this can affect the healing process. It is advisable to also avoid strenuous sporting activities until the stitches are removed.

The stitches will be removed by one of the study team at your follow-up visit approximately 10 days after your biopsy visit. If you notice any significant pain, bleeding or redness please do contact the study team for further support.

How long will I be followed up for?

We plan to follow you up for 12 months to make sure that the biopsy site heals as expected and that there is no recurrence of the Leishmania infection. If new changes developed at the healed biopsy site we might need to perform a further small biopsy to prove whether or not there was a recurrence of the Leishmania infection. If a recurrence of the leishmania infection was shown to be present in the biopsy we would give further treatment with cryotherapy. Follow up would then be extended for a further 12 months after the last cryotherapy treatment.

Would I get additional renumeration for any extra visits?

Yes, we would provide additional renumeration as follows -
Additional unscheduled visit £40
Additional minor surgery procedure £40
Small punch biopsy (4mm) diagnostic procedure £120
Cryotherapy treatment £40

What happens if there is a problem?

If you feel at any stage that there has been a problem with your participation, you can discuss this matter, in confidence, with Professor Charles Lacey, the Chief Investigator, on: tel 01904 328879 or email: charles.lacey@hyms.ac.uk

Figure 7.27: LEISH_Challenge post-biopsy patient information leaflet

LEISH_Challenge:

SAND FLY BITING STUDY

Participant Information Leaflet – Successful sand fly biting

For more information contact the project team at York:
email: vivak.parkash@york.ac.uk phone: 01904 328621

Overview

The University of York would like to thank you for taking part in the research study, "A clinical study to develop a controlled human infection model using *Leishmania major*-infected sand flies".

Your participation in this study has helped the research effort into leishmaniasis, a disease which causes around 20,000 deaths across the world every year. In particular, your efforts have assisted the scientific community in moving closer to a potential vaccine.

How did the study progress?

During follow-up, it was noted that you developed a cutaneous leishmaniasis lesion which was treated in accordance with the study protocol.

What does this mean?

This means that you developed a cutaneous leishmaniasis lesion after sand fly biting, as expected and this was treated. This was one of the potential outcomes at the start of the study that was discussed in the patient information leaflet.

After reviewing the medical literature and consulting other experts, we believe it is exceedingly unlikely that any parasites remain at the bite site with a potential to cause a cutaneous leishmaniasis lesion at this stage. Nonetheless, the study team wish to inform you of the risk of developing a lesion after being discharged from further follow-up, although it is very unlikely that a further lesion will develop especially after receiving treatment.

In this study we have used a species known as *Leishmania major* which is not known to cause severe disease, but causes a localised lesion on the skin at the site of a sand fly bite. This can appear similar in appearance to a spot. So if a further lesion did develop, it is likely that this will not spread elsewhere and is unlikely to cause long-term health effects, especially if treated.

Although there are several laboratories and 'parasite' banks throughout the world that have parasites available for use in experiments, we wanted to be able to trace the chain of these parasites and prove their safety and effectiveness. For this reason fresh parasite has been obtained from healthy individuals in Israel who have developed a *Leishmania major* lesion which was not serious, healed well and from which they have recovered fully. This specific parasite has then been tested by laboratories in Israel, Prague, Germany and York to check some of the safety aspects.

What should I do now?

There is no specific action you need to now take.

We ask however, that if you do develop any unexpected spots in the area where the sand flies were placed, that you seek medical attention. This does not need to be an emergency attendance unless you are ill and would otherwise need it.

Your GP or doctor will be able to refer you to a dermatologist (skin doctor), or an infection doctor if they feel further investigation is needed. The study may also be informed of this.

What treatment would I need?

There are a broad range of effective treatments offered for cutaneous leishmaniasis lesions, some of which were offered in the study. This can include creams applied on the lesion, injection treatments, tablet treatments, cold or heat treatments and minor surgery (as in the main study).

Would this be part of the study?

If you develop a lesion after leaving the study any further treatments will be offered via the NHS (if based in the UK), after referral from your GP.

What happens if there is a problem?

If you feel at any stage that there has been a problem with your participation, you can discuss this matter, in confidence, with Professor Charles Lacey, the Chief Investigator, on: tel 01904 328879 or email: charles.lacey@hyms.ac.uk

University of York holds Public Liability ("negligent harm") and Clinical Trial ("non-negligent harm") insurance policies which apply to this study. If you can demonstrate that you experienced serious and enduring harm as a result of your participation in this study, you may be eligible to claim compensation without having to prove that University of York is at fault. If the injury resulted from any procedure which is not part of the study, University of York will not be required to compensate you in this way. Your legal rights to claim compensation for injury where you can prove negligence are not affected. Please contact the Chief Investigator if you would like further information about the insurance arrangements which apply to the trial.

Figure 7.28: LEISH_Challenge end of study participant information leaflet – successful biting

LEISH_Challenge:

SAND FLY BITING STUDY

Participant Information Leaflet – Unsuccessful sand fly biting

For more information contact the project team at York:
email: vivak.parkash@york.ac.uk phone:01904 328621

Overview

The University of York would like to thank you for taking part in the research study, "A clinical study to develop a controlled human infection model using *Leishmania major*-infected sand flies".

Your participation in this study has helped the research effort into leishmaniasis, a disease which causes around 20,000 deaths across the world every year. In particular, your efforts have assisted the scientific community in moving closer to a potential vaccine.

How did the study progress?

After following up your case as we set out in the initial project description, we have noted that you did not go on to develop a cutaneous leishmaniasis lesion. There are many reasons for this including:

1. The sand fly may not have bitten you
2. The sand fly may not have transmitted a *Leishmania* parasite after biting
3. The *Leishmania* parasite may not have been able to cause an infection
4. The *Leishmania* parasite has become dormant

You have been followed-up to ensure that a lesion did not develop at a late stage.

What does this mean?

This means that you did not develop a cutaneous leishmaniasis lesion after sand fly biting. This was one of the potential outcomes at the start of the study that was discussed in the patient information leaflet.

From our initial FLYBITE study, we observed that not all sand flies bite human participants. We are also unable to test parasite before they are introduced to participants to determine if they contain any parasites (as this would mean killing the sand fly). Our project partners in Prague, who supplied our sand flies, have shown that not all sand flies become infected with *Leishmania* parasite even if they take a meal from infected blood. All this combined, means that some participants may not have been exposed to an infected sand fly bite. Nonetheless the follow-up period is designed to catch any possible late-stage lesions that might appear, which you have now completed.

After reviewing the medical literature and consulting other experts, we believe it is exceedingly unlikely that any parasites remain at the bite site with a potential to cause a cutaneous leishmaniasis lesion at this stage. Nonetheless, the study team wish to inform you of the risk of

developing a lesion after being discharged from further follow-up, although it is very unlikely that a further lesion will develop.

In this study we have used a species known as *Leishmania major* which is not known to cause severe disease, but causes a localised lesion on the skin at the site of a sand fly bite. This can appear similar in appearance to a spot. So if a lesion did develop, it is likely that this will not spread elsewhere and is unlikely to cause long-term health effects, especially if treated.

Although there are several laboratories and 'parasite' banks throughout the world that have parasites available for use in experiments, we wanted to be able to trace the chain of these parasites and prove their safety and effectiveness. For this reason fresh parasite has been obtained from healthy individuals in Israel who have developed a *Leishmania major* lesion which was not serious, healed well and from which they have recovered fully. This specific parasite has then been tested by laboratories in Israel, Prague, Germany and York to check some of the safety aspects.

What should I do now?

There is no specific action you need to now take.

We ask however, that if you do develop any unexpected spots in the area where the sand flies were placed, that you seek medical attention. This does not need to be an emergency attendance unless you are ill and would otherwise need it.

Your GP or doctor will be able to refer you to a dermatologist (skin doctor), or an infection doctor if they feel further investigation is needed. The study may also be informed of this.

What treatment would I need?

There are a broad range of effective treatments offered for cutaneous leishmaniasis lesions, some of which were offered in the study. This can include creams applied on the lesion, injection treatments, tablet treatments, cold or heat treatments and minor surgery (as in the main study).

Would this be part of the study?

If you develop a lesion after leaving the study any further treatments will be offered via the NHS (if based in the UK), after referral from your GP.

What happens if there is a problem?

If you feel at any stage that there has been a problem with your participation, you can discuss this matter, in confidence, with Professor Charles Lacey, the Chief Investigator, on: tel 01904 328879 or email: charles.lacey@hyms.ac.uk

University of York holds Public Liability ("negligent harm") and Clinical Trial ("non-negligent harm") insurance policies which apply to this study. If you can demonstrate that you experienced serious and enduring harm as a result of your participation in this study, you may be eligible to claim compensation without having to prove that University of York is at fault. If the injury resulted from any procedure which is not part of the study, University of York will not be required to compensate you in this way. Your legal rights to claim compensation for injury where you can prove negligence are not affected. Please contact the Chief Investigator if you would like further information about the insurance arrangements which apply to the trial.

Figure 7.29: LEISH_Challenge end of study participant information leaflet – unsuccessful biting

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Hull York Medical School
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University of York
Wentworth Way
York, YO10 5DD, UK
Direct Telephone: +44 (0) 1904 328934
Direct Facsimile: +44 (0) 1904 328844
E-Mail: vivak.parkash@york.ac.uk

[Insert GP address]

[Insert Date]

Dear Dr,

RE: A clinical study to develop a controlled human infection model using *Leishmania major*-infected sand flies

We are writing to you about [insert Volunteer Name (DOB dd/mm/yyyy)] who we understand is your patient and who has taken part in the above study. Please find enclosed a participant information sheet which describes the study in greater detail.

The participant successfully underwent sand fly bite, sustaining a cutaneous leishmaniasis lesion. This was successfully treated and the patient has completed all study follow-up.

There is only a very small risk that the participant may develop any further complications as a result of their involvement. In the unlikely scenario that this occurs, please do contact the research team (using the details above). The participant should be referred to the nearest specialist NHS Dermatology unit or the nearest NHS Infectious Diseases unit. The study team will endeavour to assist in this if necessary.

If you have any concerns or questions, please do not hesitate to contact the research team.

Yours sincerely,

Prof Charles JN Lacey
Chief Investigator

CC [insert Volunteer Name, Volunteer Address]

Figure 7.30: LEISH_Challenge end of study GP letter – successful biting

LEISH_Challenge Clinical Study Team
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Q Block
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York, YO10 5DD, UK
Direct Telephone: +44 (0) 1904 328934
Direct Facsimile: +44 (0) 1904 328844
E-Mail: vivak.parkash@york.ac.uk

[Insert GP address]

[Insert Date]

Dear Dr,

RE: A clinical study to develop a controlled human infection model using *Leishmania major*-infected sand flies

We are writing to you about [insert Volunteer Name (DOB dd/mm/yyyy)] who we understand is your patient and who has taken part in the above study. Please find enclosed a participant information sheet which describes the study in greater detail.

The participant successfully underwent sand fly bite, but did not develop a cutaneous leishmaniasis lesion. The participant was followed-up to ensure that a lesion did not develop in the expected timeframe. The literature suggests that if a lesion does not develop within the course of 6 months, then the sand fly bite was unlikely to have transmitted any parasites leading to infection.

There is only a very small risk that the participant may develop any further complications as a result of their involvement. In the unlikely scenario that this occurs, please do contact the research team (using the details above). The participant should be referred to the nearest specialist NHS Dermatology unit or the nearest NHS Infectious Diseases unit. The study team will endeavour to assist in this if necessary. If any unlikely complications do occur, it is likely that this will be in the form of a localised lesion (similar to a large spot) at the site of the sand fly bite (participants' forearm). This is very unlikely to cause any generalised (systemic) effects or spread to other regions of the body.

If you have any concerns or questions, please do not hesitate to contact the research team.

Yours sincerely,

Prof Charles JN Lacey
Chief Investigator

CC [insert Volunteer Name, Volunteer Address]

Figure 7.31: LEISH_Challenge end of study GP letter – unsuccessful biting

Appendix 2. Skin excision biopsy SOP

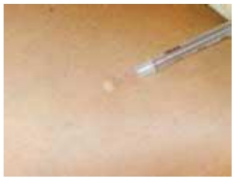
An excision biopsy of the skin is a very simple procedure performed in the outpatient setting which takes 10 to 15 minutes.

Step 1- Prepare the Area to be Biopsied:

Initially perform the study visit-specific history, examination, and image documentation. Then explain the biopsy procedures and obtain informed consent to the biopsy (suspected CL lesion), or biopsies (suspected CL lesion and matched normal skin on the contralateral arm). The informed consent also includes the subsequent study follow up. Next, the areas of skin where the biopsies are to be performed are cleaned with an alcohol swab to ensure sterile conditions.

Step 2 - Anesthetise the Skin:

The skin that is going to be removed is anesthetised by injecting a solution of 1% lignocaine and adrenaline just under the epidermis (sub-epidermally) using a 2ml syringe and orange needle. The injection continues until a “bleb” or bubble has formed under the skin greater than 4mm in diameter. The injection will burn slightly (much like a bee sting) due to a pH difference between the skin and the solution. The slight burning will quickly subside and the site will become numb.

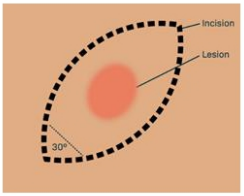


Step 3 - Check for Numbness:

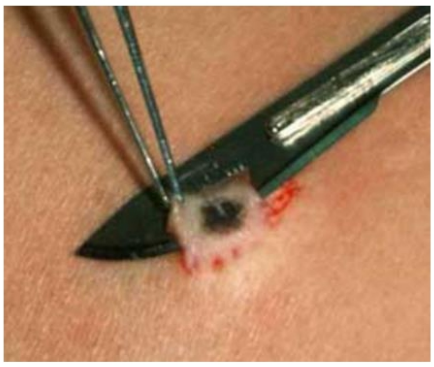
After the local anaesthetic has been administered the areas to be biopsied should be checked to ensure that the skin is properly anesthetised. A needle is used to establish whether the skin has gone numb. Great care should be taken not to force the needle into the skin. The test site should be somewhere around the periphery of the area injected. Both of these precautions ensure a viable biopsy for diagnosis later. If the patient confirms no sensation, the biopsy can proceed. A pressure sensation is normal and expected but there should be no pain. If the area requires more anaesthetic a further injection (with a new syringe) is made until the skin is completely anaesthetised.

Step 4 - Biopsy the Skin:

Once the skin is anaesthetised, an ellipse is drawn around the lesion providing a margin of normal looking skin using a marker pen. The area to be excised will be approximately 16-20 mm long, and 7-8 mm wide. The use of a length of x 2-3 of the width, with the length in the direction of Lange’s lines, provides tension free closure and optimal healing and cosmesis. A size 15 blade is used, starting at one apex and following the arc of the ellipse, whilst holding the skin in traction. The process is repeated on the other side of the ellipse.



Step 5 - Remove the Skin Biopsy: Once the elliptical incision has been made it is normal for the area to bleed. Haemostasis is achieved by direct pressure. Using toothed forceps, the tissue is carefully elevated and dissected at the level of the subcutis with the scalpel. Care is taken to dissect the tissue along even planes. Haemostasis is again achieved with pressure, or light cautery if needed. The biopsy is immediately placed in the vial containing Schneider's insect media, as below in Appendix 3.



Step 6 – Suture and Bandage the Biopsy Site: Wound closure will be achieved with 3 or 4 simple interrupted sutures using 5-0 Ethilon. The biopsy site is then covered with a small adhesive dressing.

Step 7 – Biopsy Site Care: The biopsy site should be kept clean. The site should not be submerged in water (i.e. no swimming, hot tubs, baths etc) for 48 hours. In the long term, minimal scarring may occur. In a few cases the biopsy site may form a protrusion or bump but will heal normally

Step 8 – Suture Removal

If the sutures are still *in situ* at the 10 day follow up they are removed using gentle traction on one the 'ears' and carefully cutting the 'intra-dermal loop' on the skin side of the knot, and then gently pulling the whole suture clear.

Figure 7.32: LEISH_Challenge skin excision biopsy SOP

Appendix 3. Parasitological Confirmation SOP

Parasitological confirmation will be determined using 3 different approaches, histology, culture and qPCR. All 3 approaches are performed on the skin biopsy.

Step 1 – Skin Biopsy Collection: Biopsy is collected as appendix 2, at step 6 the biopsy will be transferred to a vial containing Schneider's insect media and transferred to a biosafety cabinet.

Step 2 – Skin Biopsy Preparation: Remove the biopsy from vial using tweezers, place onto a glass slide and cut laterally in half using scalpel. Place one piece in a vial containing 4% paraformaldehyde, for histology*. Gently scrape across the surface of the remaining half collecting scrapings on glass slide, for culture. Place remaining biopsy piece in vial and immediately transfer to dry ice then -80°C, for qPCR.

Histology

Step 1 – Tissue Preparation: Tissue stored overnight at 4°C in 4% PFA. Transfer tissue to Leica tissue processor and embedding station for preparing FFPE tissue block.

Step 2 – Slide Preparation: 5µm sections will be cut from the FFPE tissue block.

Step 3 – Haematoxylin and Eosin: A slide will be deparaffinised and brought to water stained with Harris Haematoxylin followed by Eosin then dehydrated and mounted. Slides will be examined under a light microscope. Nuclei (including parasite) will be stained blue and cytoplasm and tissue structure will be stained pink.

Step 4 – Immunostaining: A slide will be deparaffinised and brought to water and antigen retrieval performed.

Then either a) or b)

a) An RNA probe specific for *Leishmania* and nuclear marker will be used. Slides will be examined by fluorescent microscopy.
b) Non-specific binding will be blocked using serum, an antibody for oligopeptidase B from *L.major* (OPB) applied followed by a fluorescently tagged secondary antibody and nuclei stain Yoyo-1.

Slides will be examined under a fluorescent microscope. Nuclei (including parasite) will be visible in one channel and parasite will be visible in the other channel.

Culture

Step 1 – Limiting Dilution Preparation: 100µl Schneider's insect media with 20% FCS will be put into 24 wells of a 96 well plate. 100µl Schneider's insect media with 20% FCS will be used to collect the biopsy scraping from the glass slide. Serial dilution will then be performed across the 24 wells. Plate will then be incubated at 26°C.

Step 2 – Limiting Dilution Reading: After 7 and 14 days the wells will be examined on an inverted microscope for parasite growth. The highest dilution with parasite growth will be noted at each time point.

qPCR

Step 1 – DNA Extraction: Biopsy portion will be weighed and extraction of total DNA will be performed using DNeasy blood and tissue kit (Qiagen) following the manufactures instructions. Tissue spiked with known parasite concentrations will used as a calibration curve.

Step 2 – Parasite quantification: SYBR green detection method will be used with primers targeting a leishmania kinetoplast minicircle DNA sequence. Results will be reported as parasites per mg tissue.

*(Further techniques as per our study objectives, not related to diagnosis, may require use of alternate media).

Figure 7.33: LEISH_Challenge Parasitological confirmation SOP

Appendix 4. Punch biopsy SOP

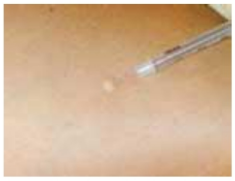
A punch biopsy of the skin is a very simple procedure performed in the outpatient setting which takes 10 to 15 minutes.

Step 1- Prepare the Area to be Biopsied:

Initially perform the study visit-specific history, examination, and image documentation. Then explain the biopsy procedures and obtain informed consent to the biopsy (suspected CL lesion), or biopsies (suspected CL lesion and matched normal skin on the contralateral arm). The informed consent also includes the subsequent study follow up. Next, the areas of skin where the biopsies are to be performed are cleaned with an alcohol swab to ensure sterile conditions.

Step 2 - Anesthetise the Skin:

The skin that is going to be removed is anesthetised by injecting a solution of 1% lignocaine and adrenaline just under the epidermis (sub-epidermally) using a 2ml syringe and orange needle. The injection continues until a “bleb” or bubble has formed under the skin greater than 4mm in diameter. The injection will burn slightly (much like a bee sting) due to a pH difference between the skin and the solution. The slight burning will quickly subside and the site will become numb.

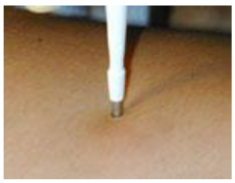


Step 3 - Check for Numbness:

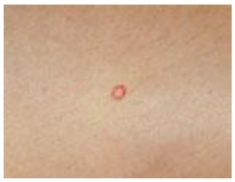
After the local anaesthetic has been administered the areas to be biopsied should be checked to ensure that the skin is properly anesthetised. A needle is used to establish whether the skin has gone numb. Great care should be taken not to force the needle into the skin. The test site should be somewhere around the periphery of the area injected. Both of these precautions ensure a viable biopsy for diagnosis later. If the patient confirms no sensation, the biopsy can proceed. A pressure sensation is normal and expected but there should be no pain. If the area requires more anaesthetic a further injection (with a new syringe) is made until the skin is completely anaesthetised.

Step 4 - Biopsy the Skin:

Once the skin is anaesthetised, using a sterile disposable 4mm skin punch perpendicular to the skin, the clinician stretches the skin using the index finger and thumb providing traction is separate directions. Using the disposable punch the clinician then applies pressure and with a firm twisting action until the blade of the skin punch has pierced the epidermis of the skin. It is normal for the patient to experience some pressure sensation but no pain. By stretching the skin the defect is oval rather than round which results in a better cosmetic result when the area is sutured.



Step 5 - Remove the Skin Punch: After the blade has sufficiently "cored" or carved out a 4mm cylinder of skin the skin punch is removed. It is normal for the area to bleed after the punch is removed. Excess blood is wiped off with sterile gauze to expose the biopsy site.



Step 6 - Excise the Biopsy: When the skin has been cored and cleared of excess blood, the next step is to remove the biopsy from the rest of the skin. Great care should be taken not to damage the epidermis by crushing it with forceps or by cutting it with a scalpel unnecessarily. The physician uses fine forceps or a skin hook to seize the dermis of the cored skin, pulls up the core to reveal dermis and subdermal fat, and uses a scalpel or scissors to snip the base of the lesion to cut the cored skin free. The biopsy is immediately placed in the vial containing Schneider's insect media, as below in Appendix 3.

Step 7 - Bandage Biopsy Site: Once the full thickness punch biopsy has been removed from the skin there will usually be some degree of bleeding which should be absorbed with sterile gauze. Haemostasis and wound closure can be achieved with 2 simple interrupted sutures using 5-0 Ethilon. The biopsy sites are then covered with small adhesive dressings.

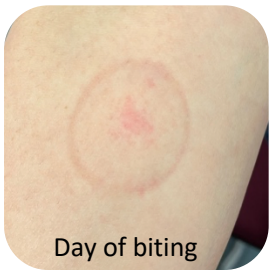
Step 8 – Biopsy Site Care: The biopsy site should be kept clean. The site should not be submerged in water (i.e. no swimming, hot tubs, baths etc) for 48 hours. In the long term, minimal scarring may occur. In a few cases the biopsy site may form a protrusion or bump but will heal normally

Step 9 – Suture Removal

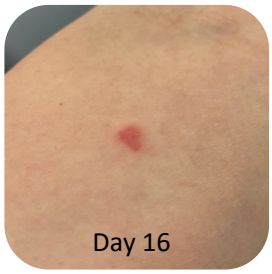
If the sutures are still *in situ* at the 10 day follow up they are removed using gentle traction on one the 'ears' and carefully cutting the 'intra-dermal loop' on the skin side of the knot, and then gently pulling the whole suture clear.

Figure 7.34: LEISH_Challenge skin punch biopsy SOP

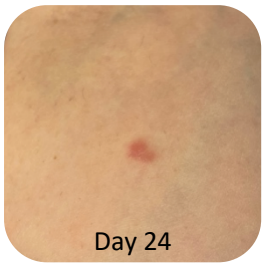
LEISH_Challenge Participant summary of lesion development
LC001

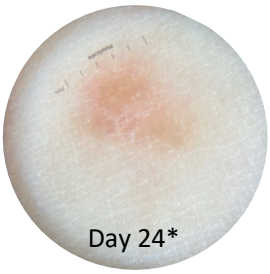

Day of biting


Day of biting*


Day 16


Day 16*


Day 24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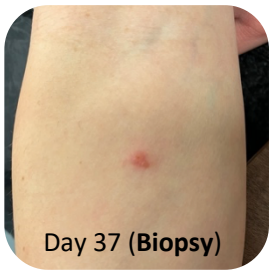
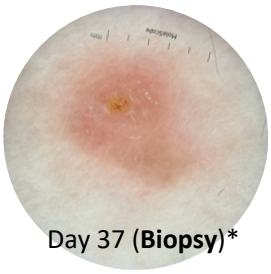
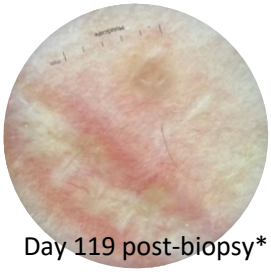
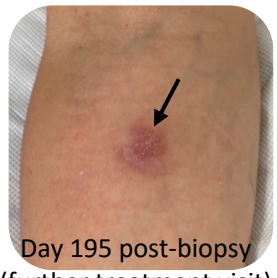

Day 37 (**Biopsy**)

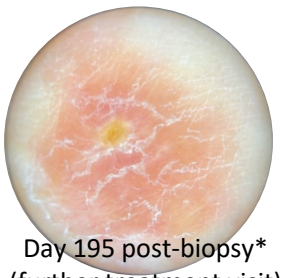

Day 37 (**Biopsy**)*

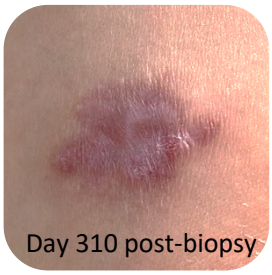

Day 56 post-biopsy


Day 119 post-biopsy


Day 119 post-biopsy*


Day 195 post-biopsy
(further treatment visit)

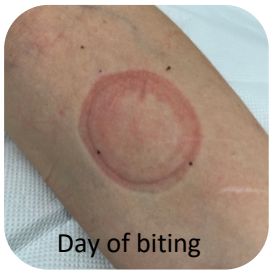

Day 195 post-biopsy*
(further treatment visit)


Day 310 post-biopsy

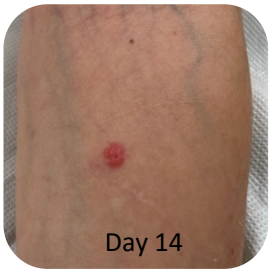

Day 434 post-biopsy

Participant summary:
6mm biting aperture used, evidence of sand fly bites and sand fly bloodmeal. Cutaneous leishmaniasis lesion development noted. 2cm x 1cm excision biopsy performed. Recurrence (indicated by black arrow), requiring diagnostic and therapeutic repeat (4mm punch) biopsy. Cryotherapy used to treat recurrence. Remains in remission.

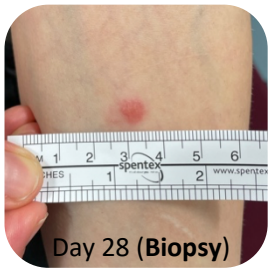
LC003

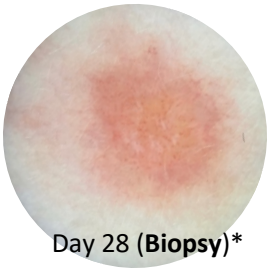

Day of biting

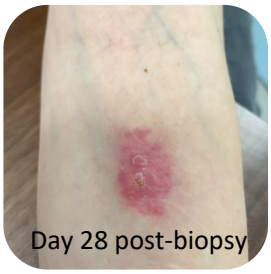

Day of biting*


Day 14

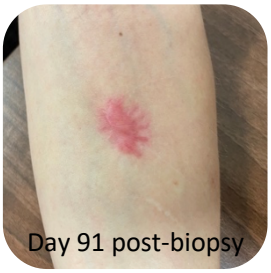

Day 14*


Day 28 (**Biopsy**)

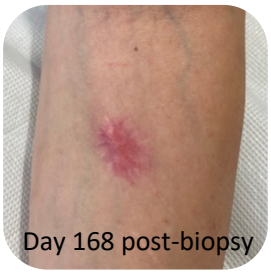

Day 28 (**Biopsy**)*

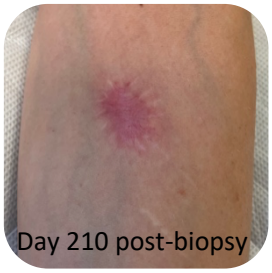

Day 28 post-biopsy


Day 62 post-biopsy


Day 91 post-biopsy


Day 154 post-biopsy

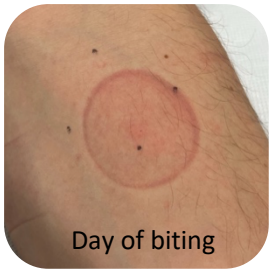

Day 168 post-biopsy


Day 210 post-biopsy

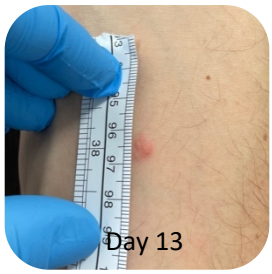

Day 335 post-biopsy

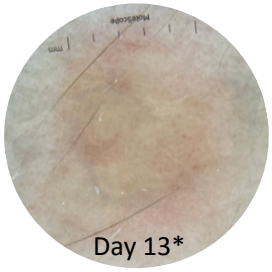
Participant summary:
6mm biting aperture used, evidence of sand fly bites and sand fly bloodmeal. Cutaneous leishmaniasis lesion development noted. 2cm x 1cm excision biopsy performed. Remains in remission.

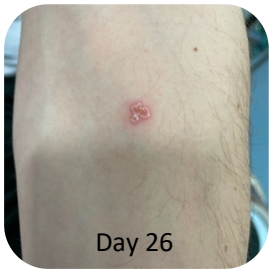
LC004

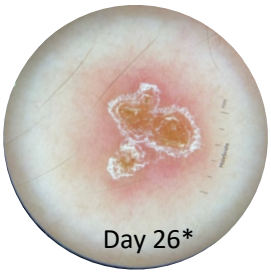

Day of biting

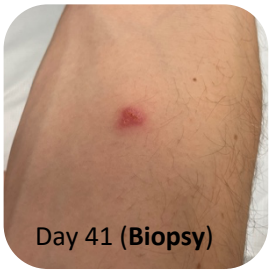

Day of biting*

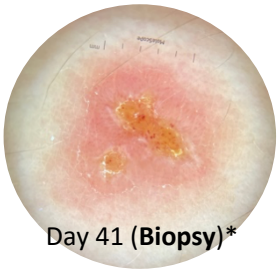

Day 13

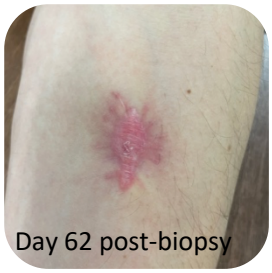

Day 13*

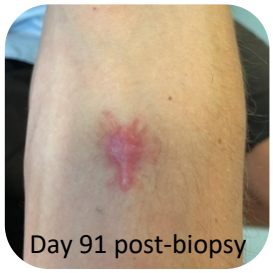

Day 26

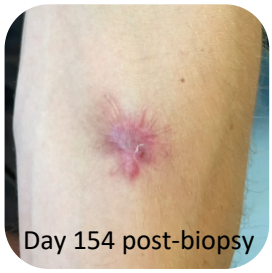

Day 26*


Day 41 (**Biopsy**)


Day 41 (**Biopsy**)*

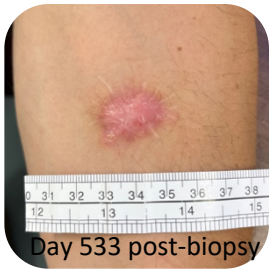

Day 62 post-biopsy


Day 91 post-biopsy


Day 154 post-biopsy

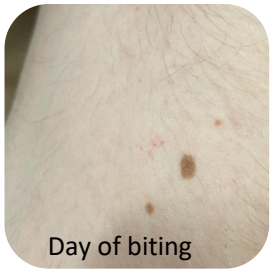

Day 175 post-biopsy
(further treatment visit)

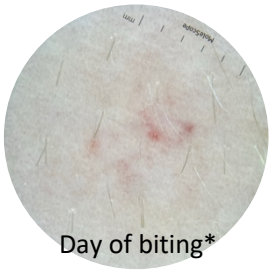

Day 175 post-biopsy*
(further treatment visit)

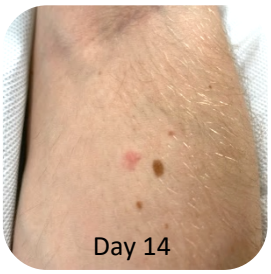

Day 533 post-biopsy

Participant summary:
6mm biting aperture used, evidence of sand fly bites and sand fly bloodmeal. Cutaneous leishmaniasis lesion development noted. 2cm x 1cm excision biopsy performed. Recurrence (indicated by black arrow), requiring diagnostic and therapeutic repeat (4mm punch) biopsy. Cryotherapy used to treat recurrence. Remains in remission.

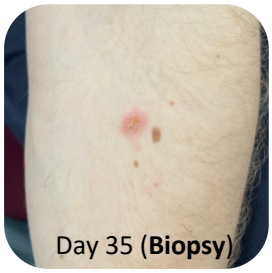
LC007

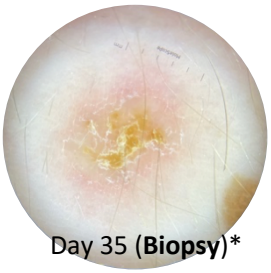

Day of biting

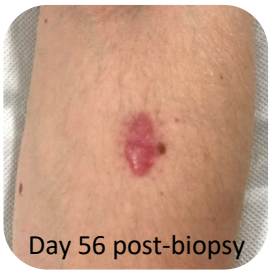

Day of biting*


Day 14


Day 14*


Day 35 (**Biopsy**)


Day 35 (**Biopsy**)*

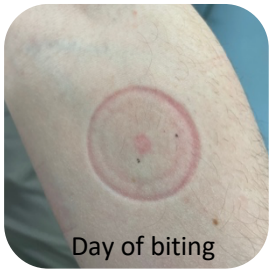

Day 56 post-biopsy

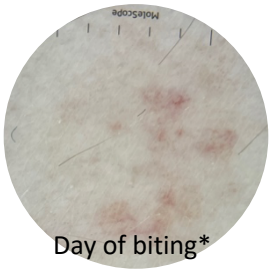

Day 111 post-biopsy

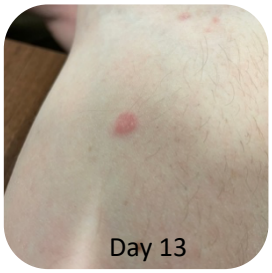

Day 194 post-biopsy

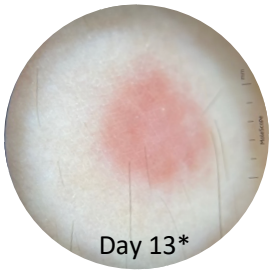
Participant summary:
6mm biting aperture used, evidence of sand fly bites and sand fly bloodmeal. Cutaneous leishmaniasis lesion development noted. 2cm x 1cm excision biopsy performed. Remains in remission.

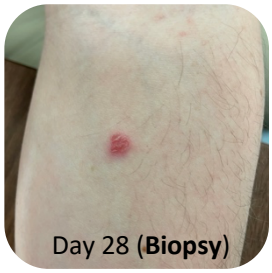
LC008


Day of biting

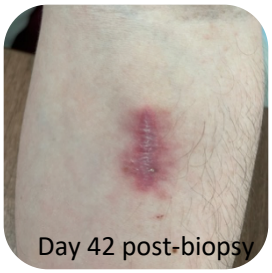

Day of biting*

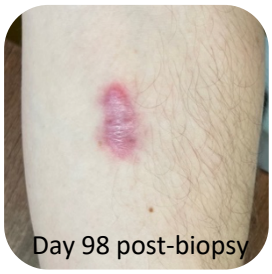

Day 13

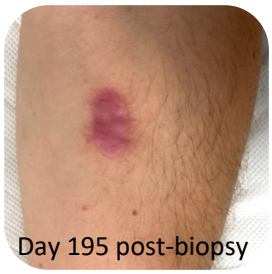

Day 13*

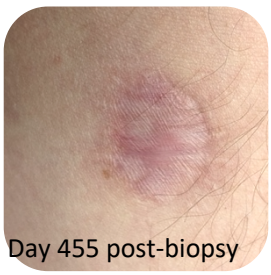

Day 28 (**Biopsy**)


Day 28 (**Biopsy**)*


Day 42 post-biopsy

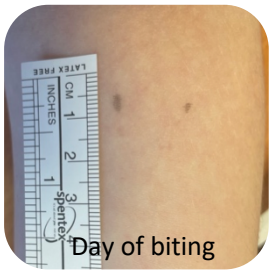

Day 98 post-biopsy


Day 195 post-biopsy


Day 455 post-biopsy

Participant summary:
6mm biting aperture used, evidence of sand fly bites and sand fly bloodmeal. Cutaneous leishmaniasis lesion development noted. 3cm x 1cm excision biopsy performed. Remains in remission.

LC012


Day of biting


Day of biting*

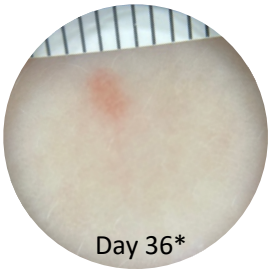

Day 12

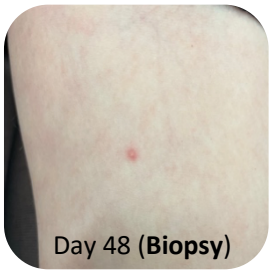

Day 12*

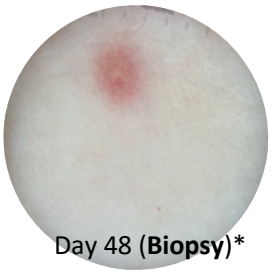

Day 26

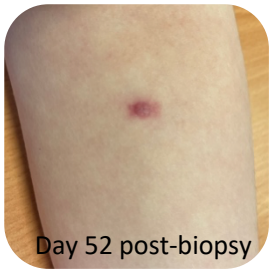

Day 26*

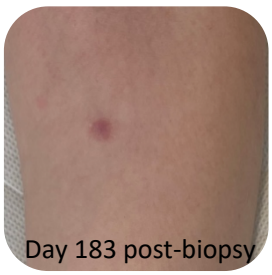

Day 36

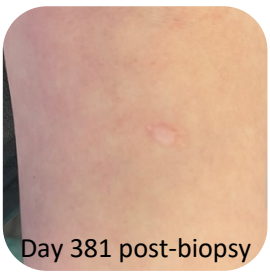

Day 36*


Day 48 (**Biopsy**)


Day 48 (**Biopsy**)*

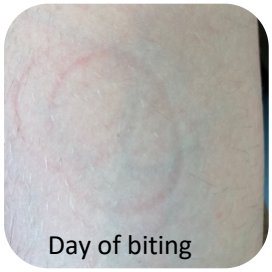

Day 52 post-biopsy


Day 183 post-biopsy


Day 381 post-biopsy

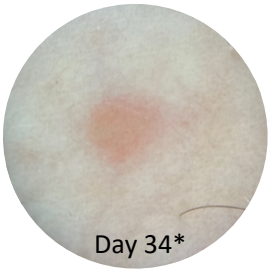
Participant summary:
6mm biting aperture used, **no** evidence of sand fly bites or sand fly bloodmeal. **No** cutaneous leishmaniasis lesion development noted. Small popular eruption noted, 5mm punch biopsy performed for diagnostics. No further lesions noted.

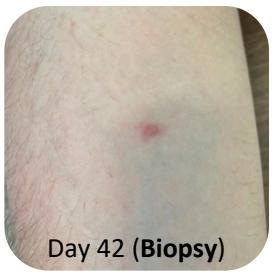
LC011

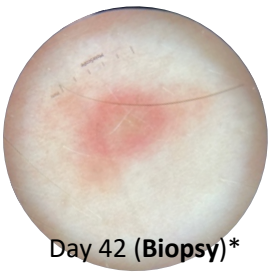

Day of biting

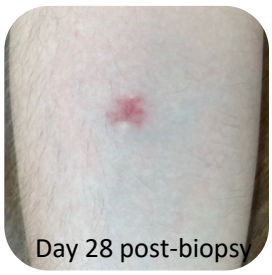

Day of biting*

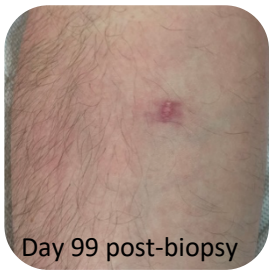

Day 34

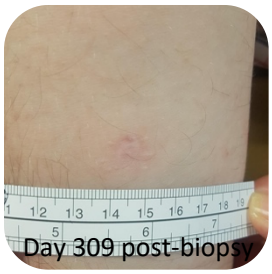

Day 34*


Day 42 (**Biopsy**)


Day 42 (**Biopsy**)*

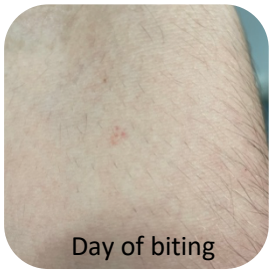

Day 28 post-biopsy

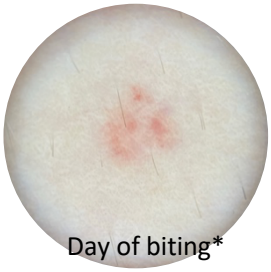

Day 99 post-biopsy

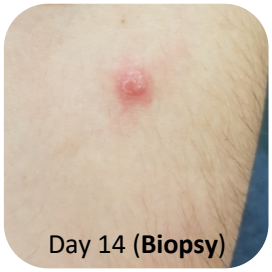

Day 309 post-biopsy

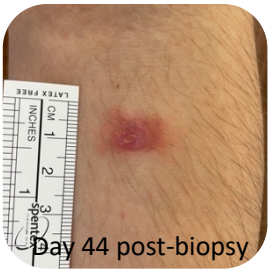
Participant summary:
4mm biting aperture used, evidence of sand fly bites and sand fly bloodmeal. Cutaneous leishmaniasis lesion development noted. 8mm punch biopsy performed. Remains in remission.

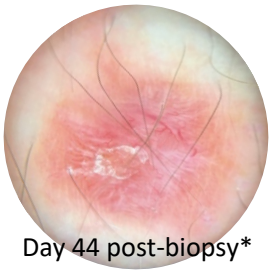
LC016

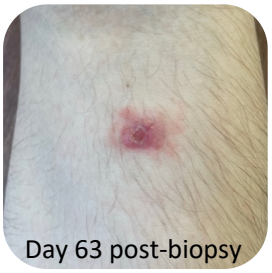

Day of biting

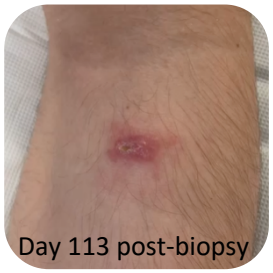

Day of biting*

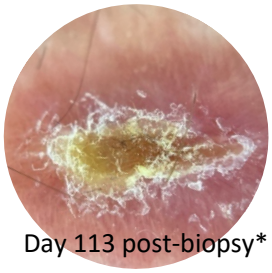

Day 14 (**Biopsy**)

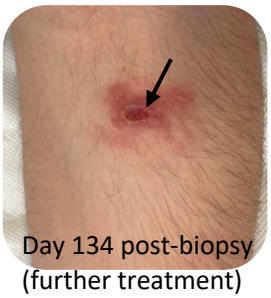

Day 44 post-biopsy


Day 44 post-biopsy*

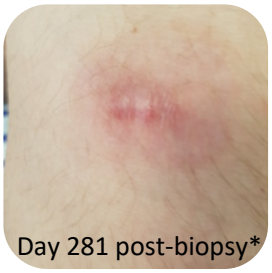

Day 63 post-biopsy


Day 113 post-biopsy


Day 113 post-biopsy*

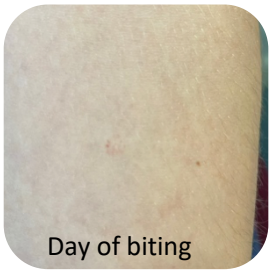

Day 134 post-biopsy
(further treatment)

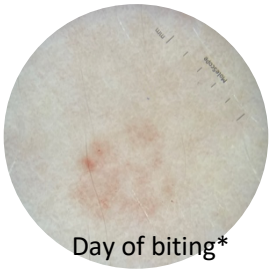

Day 134 post-biopsy*
(further treatment)

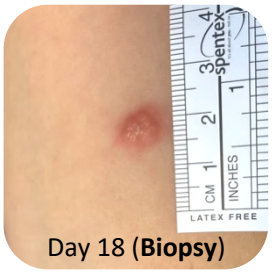

Day 281 post-biopsy*

Participant summary:
3mm biting aperture used, evidence of sand fly bites and sand fly bloodmeal. Cutaneous leishmaniasis lesion development noted. 8mm punch biopsy performed. Recurrence (indicated by black arrow), requiring diagnostic and therapeutic repeat (4mm punch) biopsy. Cryotherapy used to treat recurrence. Remains in remission.

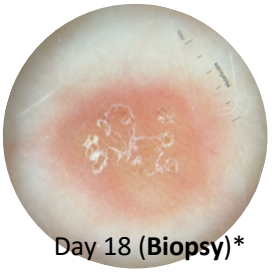
LC020


Day of biting

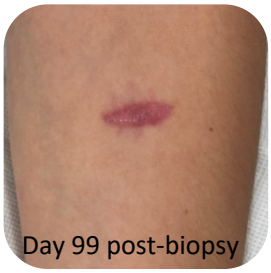

Day of biting*

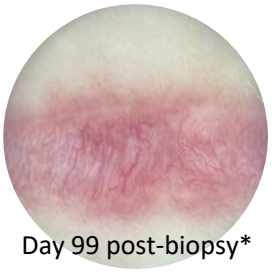


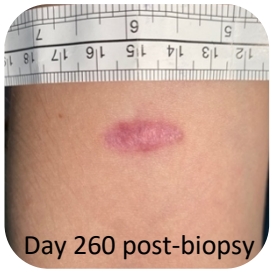
Day 18 (**Biopsy**)

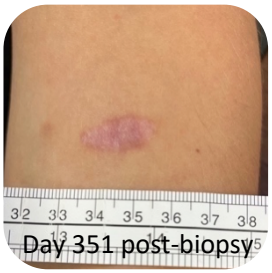

Day 18 (**Biopsy**)*


Day 29 post-biopsy


Day 99 post-biopsy

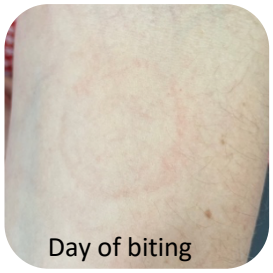

Day 99 post-biopsy*


Day 260 post-biopsy

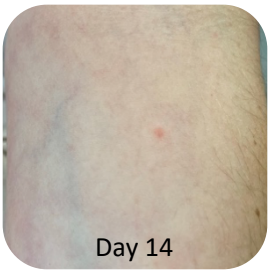

Day 351 post-biopsy

Participant summary:
5mm biting aperture used, evidence of sand fly bites and sand fly bloodmeal. Cutaneous leishmaniasis lesion development noted. 2cm x 1cm excision biopsy performed. Remains in remission.

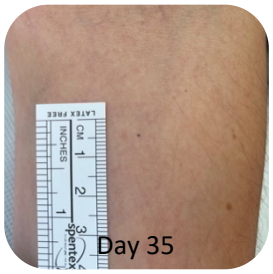
LC021

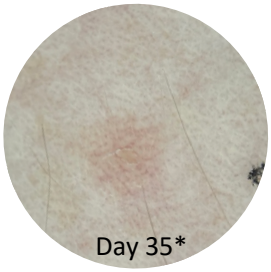

Day of biting


Day of biting*


Day 14


Day 14*


Day 35


Day 35*

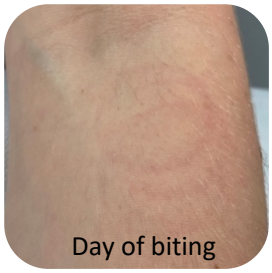

Day 64


Day 64*

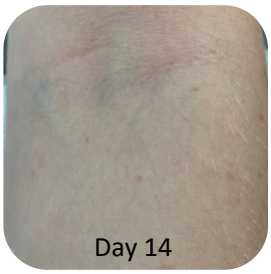

Day 156

Participant summary:
4mm biting aperture used, evidence of sand fly bites and sand fly bloodmeal. Cutaneous leishmaniasis lesion development noted. Lesion self-resolved prior to biopsy. Remains in remission.

LC023


Day of biting


Day of biting*


Day 14


Day 14*


Day 28


Day 28*


Day 70


Day 70*

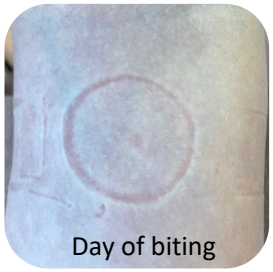

Day 100


Day 153

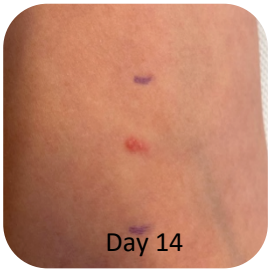

Day 153*

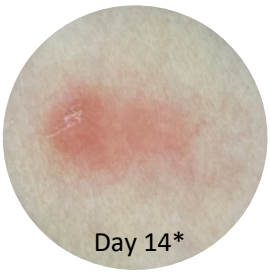
Participant summary:
4mm biting aperture used, **no** evidence of sand fly bites or sand fly bloodmeal. **No** cutaneous leishmaniasis lesion development noted. Small popular eruption noted. Lesion self-resolved prior to diagnostic biopsy. No further lesions noted.

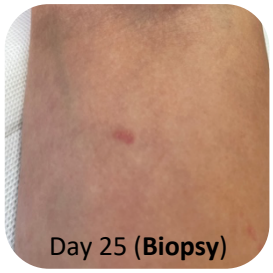
LC025


Day of biting

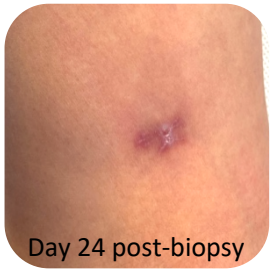

Day of biting*

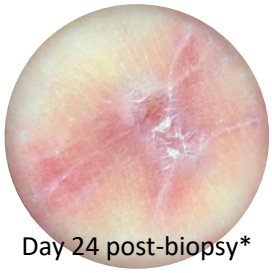

Day 14

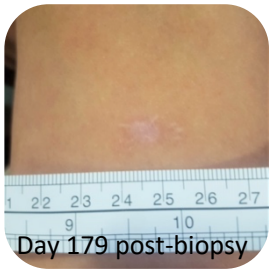

Day 14*

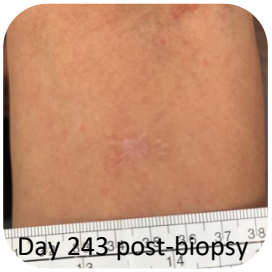

Day 25 (**Biopsy**)


Day 25 (**Biopsy**)*


Day 24 post-biopsy

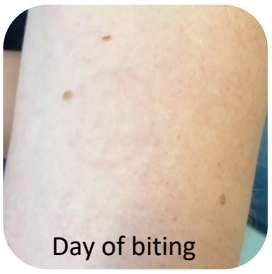

Day 24 post-biopsy*

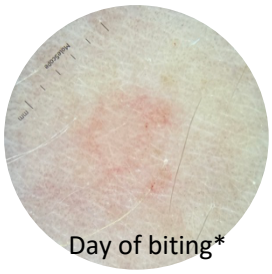

Day 179 post-biopsy

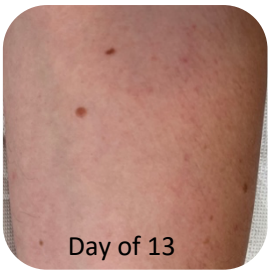

Day 243 post-biopsy

Participant summary:
5mm biting aperture used, evidence of sand fly bites but **no** evidence of sand fly bloodmeal. Cutaneous leishmaniasis lesion development noted. 8mm punch biopsy performed. Remains in remission.

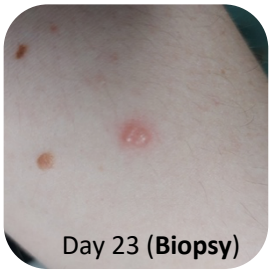
LC026

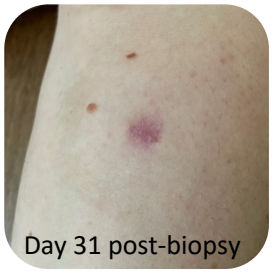

Day of biting

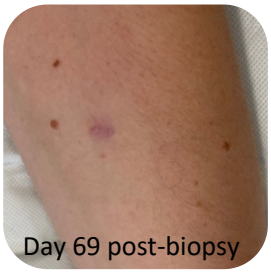

Day of biting*

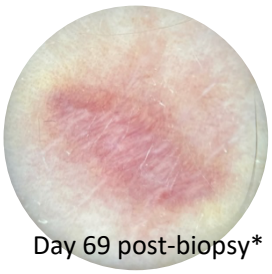

Day of 13

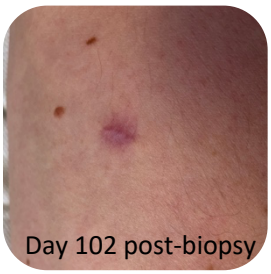

Day of 13*

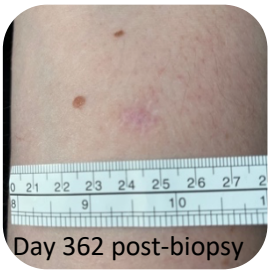

Day 23 (**Biopsy**)


Day 31 post-biopsy


Day 69 post-biopsy

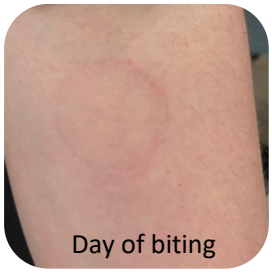

Day 69 post-biopsy*


Day 102 post-biopsy

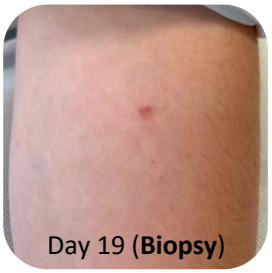

Day 362 post-biopsy

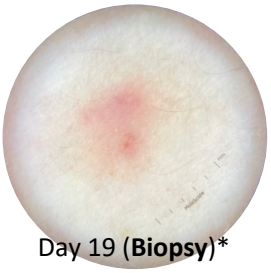
Participant summary:
4mm biting aperture used, evidence of sand fly bites and sand fly bloodmeal. Cutaneous leishmaniasis lesion development noted. 8mm punch biopsy performed. Remains in remission.

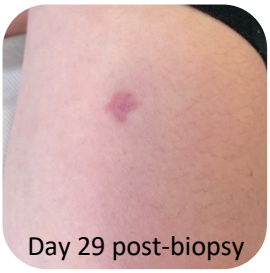
LC027

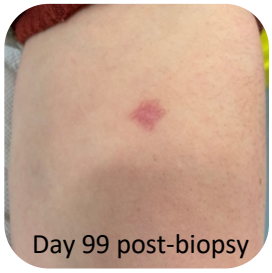

Day of biting

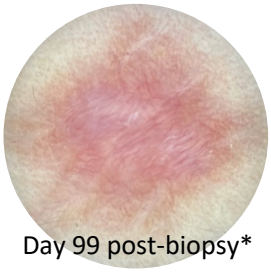

Day of biting*


Day 19 (**Biopsy**)


Day 19 (**Biopsy**)*


Day 29 post-biopsy


Day 99 post-biopsy


Day 99 post-biopsy*

Participant summary:
5mm biting aperture used, **no** evidence of sand fly bites or sand fly bloodmeal. Cutaneous leishmaniasis lesion development noted. 6mm punch biopsy performed. Remains in remission.

Figure 7.35: LEISH_Challenge picture summary for all participants

This summary depicts the lesion development for each participant during the study, including details of any primary treatment and any secondary treatment. Standard photography of lesion is given with the context of the anatomy of the arm to give a sense of size (square pictures). Where relevant, paired dermatoscopy images of lesions are given for select visits, indicated by * (circular pictures). Day of biopsy is indicated in bold. After biopsy, visits are referred to with reference from number of days since biopsy, reflecting the study plan. Recurrences where noted, are indicated with a black arrow. For dermatoscopy images, where included scale from lens is given using a millimetre scale.

SOLICITED STUDY ADVERSE REACTIONS

	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Extreme)
GENERAL				
Fever l>12 hours	37.7 - 38.9°C (100.0 – 101.5°F)	39.0 – 39.7°C (101.6 – 102.9°F)	39.8 – 40.5°C (103 - 105°F)	>40.5°C (105°F) OR max temp of >105°F
Chills/rigors	Mild hot/cold flush requires blanket or occasional aspirin/paracetamol	Limiting daily activity >6 hours, or need regular aspirin/paracetamol	Uncontrollable shaking, treatment from doctor needed	Significant medical intervention (up to and including Hospitalisation)
Malaise/abnormal tiredness	Normal activity reduced – not bad enough to go to bed	Fatigue such that ½ day in bed for 1 or 2 days	Fatigue such that in bed all day or ½ day for more than 2 days	Significant medical intervention (up to and including Hospitalisation)
General (all over) muscle aches and pains (myalgia)	No limitation of activity	Muscle tenderness, aches/pains limiting activity e.g. difficulty climbing stairs	Severe limitation e.g. can't climb stairs	Significant medical intervention (up to and including Hospitalisation)
Headache	No treatment or responds to paracetamol like treatment	Regular paracetamol like treatment needed	Regular strong painkillers needed or other medical intervention required	Significant medical intervention (up to and including Hospitalisation)
Nausea	Intake maintained	Intake reduced less than 3 days	Minimal intake 3 days or more	Significant medical intervention (up to and including Hospitalisation)
Immediate general reactions (within 6 hours of sand fly bite)			Laryngeal oedema insufficient to require intubation; diarrhoea insufficient to require IV fluids, or asthma insufficient to require hospitalisation OR Urticaria, angiooedema	Anaphylactic shock

CUTANEOUS				
Itch / pruritus	Mild itching, not requiring specific therapy	Moderate itching, requiring intermittent antihistamines	Severe itching, requiring regular antihistamines	Generalised itching, requiring on going medical management
Discomfort/pain	Mild pain that responds to paracetamol-like treatment, if needed	Pain requiring regular paracetamol-like treatment	Pain requiring regular strong painkillers	Hospitalisation
Erythema (Grades 2,3,4 can be sub-classified as macular or diffuse)	Mild erythema localised to the bite site	Moderate erythema within the aperture of the biting chamber	Diffuse erythema outside the aperture of the biting chamber but < 30% of the arm circumference	Diffuse erythema outside the aperture of the biting chamber and > 30% of the arm circumference
Blistering	Fluid filled vesicle(s) at the bite site	Fluid filled vesicle(s) within the aperture of the biting chamber	Bulla / bullae outside the aperture of the biting chamber, with no ulceration	Bulla / bullae outside the aperture of the biting chamber, with ulceration
Swelling	Palpable swelling at the bite site	Palpable swelling, within the aperture of the biting chamber	Palpable swelling outside the aperture of the biting chamber	Palpable swelling outside the aperture of the biting chamber, with subcutaneous oedema

Table 7.5: LEISH_Challenge solicited study adverse events grading