Bottling it all up: Using parasite population biology to identify susceptibility pathways in visceral leishmaniasis

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Leishmania donovani causes systemic multi-organ disease known as visceral leishmaniasis. However, little is understood about the mechanisms controlling parasite dissemination, survival and growth within and between different host tissues. Specifically, it is not known where or when parasite populations are reduced by immunological 'obstacles'; understanding this process could enable us to identify new methods to block dissemination and limit clinical disease.

CRISPR-cas9 genome editing has recently been adapted for use in *Leishmania* parasites, and used to insert genetic barcodes analysis via high-throughput sequencing, a technique termed Bar-seq. Sequence Tag-based Analysis of Microbial Population Dynamics (STAMP) has been used to determine within-host microbial pathogen dissemination patterns by comparing barcode frequencies between initial input and different host tissues at a later time-point, to calculate founder population sizes (a measure of bottleneck stringency) and genetic distance between individual populations (a measure of connectivity).

We have developed and optimised a protocol to combine these two techniques to build a library of 102 *L. donovani* lines, each containing a unique barcode. A subset of these lines (n=10) were evaluated for their growth characteristics in vitro as promastigotes and their ability to infect murine bone marrow-derived macrophages was also assessed. These studies showed comparable growth characteristics in the lines tested. Parasite location within LAMP1+ parasitophorous vacuoles was also confirmed using immunofluorescence confocal microscopy.

A pilot in vivo study was then performed by infecting BALB/c mice with 10 barcoded lines to demonstrate proof-of-concept for our methodology of barcode abundance assessment via high-throughput Illumina sequencing of PCR products from infected tissues. A second in vivo study in B6.CD45.1 mice compared PCR amplification at various time-points post-infection. Based on the results of these studies, we conclude that our approach will be suitable for analysing spatial and temporal bottlenecks at different stages of infection using STAMP.

Additionally, we will utilise genetic relatedness analysis to build a network model to describe the connectivity of parasite populations within the host. These *in vivo* results will then inform further *in vitro* studies to examine the underlying mechanisms.