Estimating complex and multiclonal infections in *Leishmania* infected patients and reservoirs

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Leishmaniasis a complex disease, comprising several parasite species, hosts, reservoirs, vectors and clinical symptoms. The clinical manifestations vary from mild cutaneous lesions to severe visceral damage, and genetic polymorphisms from both the host and the parasite have been associated with disease severity. Hybrids and genetic exchange between species and strains have already been reported for numerous parasite groups, including the *L. donovani* complex, where several species can cause visceral leishmaniasis. This has relevant implications in disease epidemiology, as it can fasten the spread of virulence and drug resistance genes. The presence of multiclonal infections, required for hybridization, could also aid in parasite adaptation to different hosts and stress conditions, as different sub-populations of the parasite could be selected in different scenarios. The presence of multiclonal infections have already been reported in some *L donovani* isolates from the Indian subcontinent and Africa using WGS and in *L. infantum* dogs in Brazil using multilocus microsatellite typing. However, the extent of multiclonal infection across several geographic locations, parasite species and hosts have not been yet estimated. In the present work, we are exploring fluctuations in allele frequency of heterozygous SNPs positions in genome sequencings of *Leishmania* isolates as a measure of multiclonal infection. The main premise is that while in clonal infections heterozygous SNPs are expected to have similar read depths in both alleles, complex multiclonal infections will disturb this proportion. We are correcting SNP calls for several confounding factors as chromosomal somy, mapping quality, call quality and read depth variations. As different *Leishmania* species/populations have different levels of heterozygosity and were sequenced in different depths, clonal simulated isolates with the same characteristics as each evaluated population were generated and used as a control. Preliminary results using ~450 whole genome sequencing of *L. infantum* and *L. donovani* isolates, from dogs and humans from Africa, Asia and Brazil have shown that a significant proportion of isolates from all sites and hosts appears to be multiclonal. We are planning on expanding this analysis to species from the *L. viannia* subspecies, to also evaluate multiclonal infections in cutaneous leishmaniasis. Finally, the proposed analysis will be packed in a framework, which could easily be adapted to other organisms.