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Superinfection with an endosymbiotic bacterium Parvibaculum lavamentivorans leads to manifest Leishmania donovani as post-kala-azar dermal leishmaniasis

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Background: Post kala-azar dermal leishmaniasis (PKDL) is a well-known dermal sequel of visceral leishmaniasis (VL) also known as kala-azar. PKDL manifests as maculo-papular skin lesions several months or years after the cure of VL, but only in 5–10% of patients. Our group has been of the opinion that PKDL is a result of *in-vivo* generation of quasi-species of *Leishmania donovani* either as *in-vivo* hybridization of various endemically circulating species within the host cells or due to super-infection with other organisms.

Material/methods: To prove this hypothesis, whole genome sequencing of recently isolated PKDL isolate was performed using 2X100bp pair-end reads on an Illumina HiSeq 2500 system and De-novo assembly of reads was constructed to longer scaffolds using A5 assembly pipeline. Using compatible bioinformatics software, pairwise alignment was performed with *Leishmania donovani* Ld2001.

Results: The sequence date produced 36.3 million reads, corresponding to more than 110 fold sequencing depth. The draft genome consisted of 1100 sequence scaffolds with a total length of 27.8Mbp and an average G+C content of 55.80%. Pairwise alignment was performed with *Leishmania donovani* Ld2001 which showed 6712 variable sites (SNPs & INDELs) in the genome of our PKDL isolate with 744 synonymous, 1068 non-synonymous SNPs and 20 frame-shift INDELs. Approximately 138 tRNAs were identified in the genome, which are little higher than other Leishmania isolates. Sequence reads when mapped using BWA v0.7.10, Only 2-5% reads were mapped with *L. infantum* (4.2%), *L.major* (4.6%), and *L. braziliensis* (1.8%). The genome of our isolate showed >98.0% homology to the Indian strains *L. donovani* Ld 2001, *L. donovani* Ld 39 and *L. donovani* BHU-109. The BLAST analysis suggested that the 11,281 sequence reads had homology with *Leptomonas seymouri* as reported earlier also but we also found 893 contigs of a heterotrophic bacterium *Parvibaculum lavamentivorans DS*-1 genome (GenBank accession no. LBGS00000000.2).

Conclusions: The de-novo sequencing done for the first time, indicated a possibility of endosymbiotic infection of the Leishmania donovani with a bacterium *Parvibaculum lavamentivorans* leading to PKDL manifestations of leishmaniasis only in a small percentage of patients.