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De novo transcriptome sequencing of two *Leishmania tropica* strains positive and negative for *Leishmania* RNA virus 2 and differential gene expression analysis of 30 isolates from Turkey

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Background
Leishmania RNA virus (LRV), is found as an endosymbiotic in the cytoplasm of *Leishmania* parasites affecting the parasite virulence and disease pathogenesis. Virus-induced inflammatory cascade can increase disease severity, parasite persistence, and anti-leishmanial drug resistance [1,2]. However, there are no studies at the transcriptomic level that functionally investigate the relationship between *Leishmania*-virus-host and its mechanisms of action in The Old World. In this study, we aimed to investigate the pathogenic mechanisms of LRV2 +/- *Leishmania* strains isolated from human and canine leishmaniasis cases.

Methods
The transcriptomic sequencing of two *L. tropica* (LRV2 +/-) strains (MHOM/TR/03/EP90 & MHOM/TR/04/EP94) isolated from CL patients were performed. A sequencing library for RNA samples was prepared via the BGISEQ-500 transcriptome library workflow according to the manufacturer's protocol to obtain clean reads; low-quality reads, reads with adapters and reads with unknown bases were filtered out. A BLASTx search was performed against protein databases, including the KOG (euKaryotic Orthologous Group database), Nr (non-redundant protein) protein database [3], KEGG (Kyoto Encyclopedia of Genes and Genomes protein database), and SwissProt to determine the functional annotation of unigenes. The Blast2GO [4] and InterProScan5 programs with Nr annotation were used to acquire the GO (Gene ontology) and InterPro annotation of unigenes, respectively.
Bioinformatic analyses identified 6774 genes as differentially expressed genes (DEGs). Among these, 33 DEGs were selected for validation testing. qRT-PCR was performed to determine these gene expression levels in 30 *Leishmania* strains including *L. infantum* (causative of CanL), *L. tropica* (causative of CanL and CL), and *L. major* (causative of CL). After performing PCR, Ct values were used to calculate close fold changes by applying the improved 2-ΔΔCT method with efficiency correction.

Results
Bioinformatic analyses identified 5023 up-regulated and 1751 down-regulated genes as differentially expressed genes (DEGs). We annotated the Unigenes with the KOG database and calculated the Unigene distribution for 25 functional groups. Based on the GO classification and functional enrichment, 7792 unigenes were classified into three main independent categories (cell components, molecular functions and biological processes) and 53 sub-categories. Unichanges were selected if they could be related to *Leishmania* and LRV2 interaction mechanisms and showed significant fold change.
Statistically significantly expressed genes were obtained in the LRV2+ *L. tropica*, related to intracellular parasite survival, transcription, translation, signal transduction, biosynthetic process, parasite proliferation and mechanisms of action on host cells. Twenty-five genes of 33 DEGs in the samples of CanL and human leishmaniasis cases were consistent with bioinformatic analysis of transcriptomic sequencing. Notably, the genes with the highest fold value were fatty acid elongase 2, snare protein, histone 4, gp63, ppgi, signalling mucin MSB2, cytosolic triparedoxin peroxidase and DNA repair protein alkB.

Conclusions
This study revealed for the first time the functional annotation of LRV2+/- *L. tropica* strains at the RNA level in The Old World *Leishmania* by *de novo* RNA-Seq. Although some DEGs obtained in this study are well

known, their mechanism of action in the *Leishmania*-virus-host relationship is not fully understood [1,2]. DEGs were mostly associated with defence, signalling, reproduction and survival mechanisms [5]. In particular, some DEGs may be used as diagnostic biomarkers in both human and canine leishmaniasis. Moreover, the results obtained from this study identified molecules that may be related to the effect of LRVs on human/canine leishmaniasis pathogenicity and could be used to develop recombinant vaccines and/or immunotherapies containing immunogenic components.

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