Validation of a leishmania RNA virus-1 detection assay in a non-endemic setting

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Background: Tegumentary leishmaniasis is an emerging disease among travelers, and case reports suggest that TL among travelers may be particularly severe. Leishmania RNA virus-1 (LRV1) has been implicated in the pathogenesis of severe TL in murine models, though whether this extends to humans is unknown. We sought to validate a LRV-1 detection assay.

Methods & Materials: ATCC reference and clinical strains of Leishmania identified to species level were screened for LRV1. Two real time PCR assays for detection of LRV1 were performed with LRV1 set A and set B primers. Amplification of Leishmania kinetoplastid membrane protein 11 (kmp11) served as a quantification and extraction control. ATCC L. (V.) guyanensis strain MHOM/BR/75/M4147 was used as a positive control.

Results: We screened 5 New World ATCC strains of Leishmania (L. amazonensis, L. mexicana, L. (V.) braziliensis, L. (V.) panamensis, and L. (V.) guyanensis) and 12 clinical strains from primary clinical samples including: bone marrow (N = 1), whole blood (N = 2), skin scraping (N = 2), skin biopsy (N = 2), cytology brush (N = 4) and unspecified (N = 1). Causative Leishmania species in clinical specimens were as follows: L. chagasi/infantum (N = 3), L. (V.) braziliensis (N = 3), L. tropica (N = 2), L. (V.) panamensis (N = 1), L. major (N = 1), and L. (V.) guyanensis (N = 1). Amplification of LRV1 primer sets A and B along with kmp11 only occurred with the ATCC strain of L. (V.) guyanensis known to harbour LRV1. Kmp11 alone was amplified in the remaining ATCC strains, and in 6 of 12 (50%) clinical strains, thus 80% of ATCC strains and 50% of clinical strains could be deemed negative for LRV1. Neither LRV1 setA or set B primers nor kmp11 were amplified in 6 of 12 (50%) clinical samples, leading to an indeterminant result regarding LRV1 co-infection status.

Conclusion: Detection of LRV1 from clinical strains of Leishmania may become relevant to clinicians in the future. Performance of the LRV1 assay was superior in cultured ATCC strains, which reflects high parasite concentrations in culture compared to primary clinical specimens. In 50% of clinical isolates, we were able to assign an LRV1 result, thus, further optimization of assay performance in primary clinical specimens is warranted.

http://dx.doi.org/10.1016/j.ijid.2014.03.760

Validation of a molecular diagnostic algorithm for leishmania detection in a non-endemic setting

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Background: Tegumentary leishmaniasis is an emerging disease among travelers. Diagnosis of TL rests on demonstration of parasites by culture or microscopy, both of which are insensitive and operator dependent. We validated a molecular diagnostic algorithm for Leishmania detection and species identification using clinical specimens.

Methods & Materials: Microscopy, culture, and Leishmania genus 18S real time PCR were performed on clinical specimens sent for Leishmania detection between May 2012 and October 2013. Specimens positive by 18S PCR were subjected to ITS1 end-point PCR followed by restriction fragment length polymorphism (RFLP), which identifies L. donovani, L. chagasi/infantum, L. major, L. tropica, L. amazonensis, and L. mexicana. For the Viannia sub-genus, amplification of the ITS1 region differentiates L. (V.) braziliensis from the other species within the complex. Species identification was confirmed by Sanger sequencing of the ITS2 region or by a combination of Mpi (mannose phosphate isomerase), Cpb (cysteine proteinase b), and Hsp70 (heat shock protein-70) PCR-RFLP/Sanger sequencing for L. Viannia complex.

Results: Forty-five specimens from 36 individuals were tested for Leishmania during the study period, and of these, 20 specimens from 14 individuals were positive. Primary specimens were as follows: bone marrow (N = 5), organ (N = 2), whole blood (N = 3), skin scraping (N = 5), skin biopsy (N = 10), skin aspirate (N = 9), filter paper (N = 3), cytology brush (N = 5) and unspecified (N = 3). Compared to 18S PCR (gold standard), culture had a sensitivity of 15% and specificity of 100%, while microscopy had a sensitivity of 27% and specificity of 100%. Among the 14 individuals with confirmed Leishmania infection, causative species were as follows: L. tropica (N = 3), L. (V.) panamensis (N = 3), L. chagasi/infantum (N = 2), L. major (N = 2), L. (V.) braziliensis (N = 2), and L. (V.) guyanensis (N = 1). Turnaround time of 18S and ITS1 PCR is 2 days, while 18S PCR followed by PCR-RFLP or ITS2 sequencing is 4 days.

Conclusion: Using a combination of end-point and real time PCR, along with RFLP and sequencing, we have demonstrated reliable detection and species identification of Leishmania from a range of primary clinical specimens. The utility of culture and microscopy