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

R. Lau[1], B. Valencia[2], F. Ralevski[1], A. Llanos-Cuentas[3], A.K. Boggild[4,*]

[1] *Public Health Ontario Laboratories, Toronto, Canada*
[2] *Institute of Tropicale Medicine "Alexander von Humboldt", Lima, Peru*
[3] *Institute of Tropical Medicine "Alexander von Humboldt", Lima, Peru*
[4] *Tropical Disease Unit, Division of Infectious Diseases, University Health Network-Toronto General Hospital, Toronto, Canada*

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 B primers nor kmp11 were amplified in 6 of 12 (50%) clinical strains, 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.

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

R. Lau[1], B. Valencia[2], F. Ralevski[1], A. Llanos-Cuentas[3], A.K. Boggild[4,*]

[1] *Public Health Ontario Laboratories, Toronto, Canada*
[2] *Institute of Tropicale Medicine "Alexander von Humboldt", Lima, Peru*
[3] *Institute of Tropical Medicine "Alexander von Humboldt", Lima, Peru*
[4] *Tropical Disease Unit, Division of Infectious Diseases, University Health Network-Toronto General Hospital, Toronto, Canada*

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

is limited by poor sensitivity, technical expertise, and in the case of culture, slow turnaround time.

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Case report: A rarely seen cause of brain abscess - neurotoxocariasis

O.R. Sipahi [1], E. Erdem Kivrak [1], M. Korkmaz [1], M. Tasbakan [2], H. Pullukcu [2], B. Arda [1], T. Yamazhan [1], S. Ulusoy [1,*]

[1] *Ege University-Faculty of Medicine, Izmir, Turkey*
[2] *Ege University Medical Faculty, Izmir, Turkey*

Background: Toxocariasis is associated with three syndromes in human beings which are visceral larva migrans, ocular larva migrans and covert toxocariasis. Although neurotoxocariasis is defined as the fourth syndrome of toxocariasis, it is usually considered as a neurological disease which is usually concomitant with visceral larva migrans. İn this abstract we report a case of brain abcess caused by toxocariasis.

Methods & Materials: A 56 years-old female patient admitted at our hospital with headache, pain referring to right side of her face and teeth, numbness of forth and fifth finger of her right hand. Cranial diffusion weighted, dynamic magnetic resonance imaging (MRI) revealed a few non- spesific intensities at supratentorial white matter, and an approximately 13x12 mm lesion without contrast enhancement which had a significant edema around the white matter in the left frontal cortex. Histologic examination after stereotactic biopsy of the lesion revealed diffuse histiocyte infiltration in histological examination.

Results: A spesific agent could not be detected in histochemical examination. Western-blot test toxocariasis in serum and CSF were positive. She was transferred to the infectious diseases and clinical microbiology clinic.Albendazole 400 mg q12 h was started. A total of one month duration for albendazole treatment was planned after regression of the cranial MRI findings on the 14th day of therapy. The patient is recalled for cranial MRI control three months later. However, we found out that she continued albendazole for three months. Compared to the previous MRI, there were two stabilized T2A hyperintense lesions in left cranial hemisphere and minimally regressed lesions at the level of left frontal centrum semi-ovale. There was no relapse after six month follow up. The

Conclusion: The presented case suggests that although rarely neurotoxocariasis may be encountered in the etiology of encephalitis/brain abcess. The patient was successfully treated with albendazole. To our knowledge this is the first reported case of neurotoxocariasis n Turkey.

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The influence of winter on the prevalence of urogenital schistosomiasis in rural southern KwaZulu-Natal

E.S. Christensen [1,*], M. Taylor [2], S. Gagai [2], S. Zulu [2], K. Lillebø [1], S.G. Gundersen [3], S.D. Holmen [1], E. Kleppa [1], E.F. Kjetland [2]

[1] *Oslo University Hospital, Oslo, Norway*
[2] *University of KwaZulu-Natal, Durban, South Africa*
[3] *Sorlandet Hospital, Kristansand, Norway*

Background: The WHO recommends that praziquantel is administered to all children above four years of age living in schistosoma endemic areas. The frequency of mass drug administration depends on the community's prevalence level. Urine egg count remains the gold standard method for measuring prevalence levels and it is usually done immediately prior to mass treatment. This study aims to explore the influence of seasons on urine egg excretion.

Methods & Materials: In a schistosomiasis endemic area in southern KwaZulu-Natal, South Africa, where the climate is characterized by hot and humid summers (high-transmission-season) and cool, dry winters (low-transmission-season) two cross-sectional studies were performed in randomly selected schools: (1) February to November (2009, 2010 or 2011) urines were collected from females aged 10-12 and 16-23 years (n = 1988), (2) May to September 2011 urines were collected from randomly selected school girls aged 4-23 years (n = 2371). No mass-treatment had been performed prior to the surveys. Urines were examined for *S. haematobium* eggs by microscopy.

Results: In the 4359 urine samples from females aged 4-23 years (mean 13.35, SD 4.1) 24.6% were *S. haematobium* positive. The number of female pupils with eggs in urine fluctuated in parallel with the transmission seasons. The prevalence was significantly higher immediately after the high-transmission season, summer, 37.8% positive, than in the low-transmission-season, winter, 14.5% positive (OR 0.68, 95%CI 0.62-0.76, $p < 0.01$). There was no influence of age on the association. Likewise, the intensity of infection in the positive girls was significantly lower in winter (Mann-Whitney U Test, $p = 0.049$).

Conclusion: This study shows that the number of subjects excreting *S. haematobium* ova in urine varies by season. The *S. haematobium* prevalence was highest immediately after the high-transmission season. In our study area urines are best collected shortly after the hottest season in order to make decisions about eligibility for mass-treatment, whereas treatment is known to be most efficient when given in the cool season. Further studies are needed to confirm whether egg excretion has to do with factors such as temperature, high-transmission, rainfall, parasite fecundity, or intensified water activity.

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