SPECIAL ISSUE:
SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

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I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS
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José Luís Capelo Martínez, PhD. FRSC
LAQV-REQUIMTE, Chemistry Department, Universidade NOVA de Lisboa, Portugal.

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LAQV-REQUIMTE, Chemistry Department, Universidade NOVA de Lisboa, Portugal.
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New World cutaneous leishmaniasis in a traveller to a country endemic for Old World cutaneous leishmaniasis

Molecular identification of Leishmania species in pediatric population attended at the National Institute of Health

Aide Sandoval-Juarez 1, Gloria Minaya-Gómez 1, Nyshon Rojas-Palomino 1, L F Donaires 1.

1 Instituto Nacional de Salud—Perú.

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ABSTRACT

Leishmaniasis is a parasitic disease that affect majority of people of endemic area mainly to the pediatric population, and is caused by a parasite protozoa of the Leishmania genus. In children, the identification of Leishmania species infecting is very important, because it allows establishing criteria and protocols for will prevent the progression of disease to more aggressive clinical forms, as mucocutaneous and diffuse leishmaniasis. Our aim was to identify the Leishmania species in samples obtained from 30 children (between 1 and 14 years old), who were attended to the Peruvian National Institute of Health and parasitologically confirmed as American Tegumentary Leishmaniasis. The identification of Leishmania species, were development by High Resolution Melting Analyzing (HRMA) of the conserved region of the kDNA Leishmania. Initially, we extracted parasitic DNA from 17 Leishmania spp strains isolated by in vitro Culture and 13 smear Giemsa-stain with positive result by microscopy visualization of amastigotes. Later, we amplified the conserved region of the kDNA Leishmania parasitic DNA through PCR-High Resolution Melting. The data was recorded and analyzed by RotorGene Q software and Stata 13.0.v. The Median age of the 30 children was 7.5 years old (Interquartile range [ICR]: 3-11) and 60% (18/30) were male. The median time of disease was two months (ICR:1-4), 83.3% (25/30) of those evaluated had a single lesion, with an average surface area of 4.8 cm². (ICR:0.7-5). The identified species were Leishmania (Viannia) peruviana, (40%), Leishmania (V.) guyanensis (27%), Leishmania (V.) braziliensis (20%), Leishmania (V.) lainsoni (10%) and Leishmania (Leishmania) amazonensis (3%). We found three cases of mucocutaneous leishmaniasis from Huánuco, Ayacucho and Cusco, that were caused by L. (V.) braziliensis, L. (L.) amazonensis and L. (V.) guyanensis, respectively. Also, our found four cases of Leishmaniasis recidivans of children from La Libertad, Lambayeque and Pasco. In Peru, the information related to Leishmaniasis is inadequate, similar occur with information related to Leishmaniasis in children population that is scarce, because the notification just consider general aspect of the confirmed cases, without adequate disaggregation by age , social status, among other. In other hand, we achieves identify Leishmania strains isolated from children as Leishmania (V.) braziliensis and Leishmania (V.) guyanensis from areas where not been previously reported. We recommend continuing studies of identification of Leishmania species in samples from children population that involve a greater number of participants, also develop the validation of Leishmania species identification through of HRMA of the conserved region of kDNA in compare with gold standard methods or Cytochrome B gene sequencing, and continuing studies related to dispersion of Leishmania species to new endemic areas.

References:

Correspondence: Email - asandoval@ins.gob.pe
Fluoroquinolones: an ancient antibiotic potentially useful against leishmaniasis

Ana Poveda 1*, Javier Santamaría-Aguirre 1, Lissette Esteves 1, Luis Sebastián Espíndola 2, Gina Loachamin 1, Karen Tufío 1, Gabriela Morales 3, Eliana Lara 3 and Miguel Ángel Méndez 2.

1Instituto de Investigación en Salud Pública y Zoonosis, Facultad de Ciencias Químicas, Universidad Central del Ecuador, Quito; 2Grupo de Química Computacional y Teoría Universidad San Francisco de Quito.

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Abstract

Leishmaniasis is a largely neglected infection caused by Leishmania spp. parasites. The first-line treatment, antimonate meglumine, has a large number of adverse effects, high costs and is developing resistance. New alternatives are mandatory. An interesting target is topoisomerases type II, that conserve the critical homologous serine residue inhibited by fluoroquinolones [1]. They have lower cost and fewer adverse effects. To determine the leishmanicidal effect of fluoroquinolones, a fluorescence method was optimized to determine MIC and IC50 in cultures of L. mexicana and L. braziliensis promastigotes. Our results show a good leishmanicidal activity of fluoroquinolones, being enrofloxacin the most effective. Protein modelling and docking results are consistent and supports that these enzymes are targets of fluoroquinolones. Leishmania parasites have two genes encoding topoisomerases type II, one located at the nucleus and the other in the mitochondria. Pulse field gel electrophoresis (PFGE) and mitopotential suggest that the mitochondrial enzyme is the main target. Enrofloxacin is ionized in a wide range of pH, limiting its absorption through the biological membranes. This limitation could be solved loading it into liposomal systems such as the transfersomes, ultraformable nanovesicles. Transfersomes were characterized in terms of size, polydispersity index, zeta potential, entrapment percentage, dissolution profile and physical stability. These nanovehicles enhanced the leishmanicidal activity compared with enrofloxacin in solution, around 15 times. So the nanoencapsulation could be an interesting approach to develop a topic formulation to treat cutaneous leishmaniasis.

Figure 1 | A. Modelled 3D structure of mtTopOll; B. AFM image of transfersomes and chemical structure of enrofloxacin, C. MIC and IC50 of transfersomes, enrofloxacin and meglumine sb.

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References:

Correspondence: Email - apoveda@uce.edu.ec
Leishmania extracellular antigens properties and future applications

N Santarém ¹, A Cordeiro-da-Silva ², ²*

¹ Parasite Disease Group, IBMC - Instituto de Biologia Celular e Molecular, Portugal; ² Faculdade de Farmácia, UP - Universidade do Porto, Portugal.

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Abstract

The use of secretion pathways for effector molecule delivery is an essential part of the millennia struggle between pathogens and their host immune system. Leishmania protozoa are no exception. Recent emphasis has been given to the parasite released extracellular vesicles (EVs) as important agents in this process. Still, these EVs are not alone as players and their significance in the context of other Leishmania secreted antigens is still to be observed. To address this we evaluated the immunological potential of the promastigote exoproteome (EXO) and its associated components EVs and vesicle depleted exoproteome (VDE). We also evaluated the potential use of these antigens for serodiagnosis.

In an air pouch model, a dose-dependent recruitment of immune cells was observed for all the exoproteome components. Interestingly, EVs and VDE induced a different recruitment compared with parasites, attracting significantly more dendritic cells, which remain non-activated at the site of inoculation, and fewer neutrophils. Interestingly in vitro, EVs, VDE, and EXO, as well as the parasites, were able to diminish the response capacity of DCs and macrophages to TLR ligands suggesting that these components interfere with functional aspects of these cells. Overall the secreted antigens conferred a real as they increase the parasite burden in a dose-dependent manner. In the future, these exogenous antigens can be a source of biomarkers for diagnostic and vaccine use.

Correspondence: Email - cordeiro@ibmc.up.pt
Exploring the effect of a sandfly salivary protein “prime” on the immunogenicity of a complex vaccine formulation containing both vector and parasite-derived antigens

Pedro Cecílio 1, 2, *, Begoña Pérez-Cabezas 1, 2, Laura Fernández 4, Javier Moreno 4, Eugenia Carrillo 6, José M. Requena 2, Epifanio Fichera 6, Steven G. Reed 7, Rhea N. Coler 7, Shaden Kamhawi 6, Fabiano Oliveira 8, Jesus G. Valenzuela 8, Luigi Gradoni 9, Reinhard Glueck 5, Gaurav Gupta 3 and Anabela Cordeiro-da-Silva 1, 2, 3.

1 Parasite Disease group, Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Porto, Portugal; 2 IBMC - Instituto de Biologia Celular e Molecular, Universidade do Porto, Porto, Portugal; 3 Faculdade de Farmácia da Universidade do Porto, Porto, Portugal; 4 WHO Collaborating Centre for Leishmaniasis, Centro Nacional de Microbiologia, Instituto de Salud Carlos III, Madrid, Spain; 5 Centro de Biologia Molecular Severo Ochoa (CSIC-UAM), Universidad Autónoma de Madrid, Madrid, Spain; 6 Etna Biotech S.R.L, via Vincenzo Lancia, 57 - Zona Industriale Blocco Palma 1, Catania, Italy; 7 Infectious Disease Research Institute (IDRI) , Seattle, WA, USA; 8 Vector Molecular Biology Section, Laboratory of Malaria and Vector Research, NIAID, NIH, Rockville, MD, USA; 9 Unit of Vector-borne Diseases and International Health, Istituto Superiore di Sanità, Rome, Italy.

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Abstract

Although vaccination is accepted as a potentially effective approach to prevent leishmaniasis, to date there is no vaccine available for human disease. Leishmania parasites are transmitted by sandflies from the genus Lutzomyia and Phlebotomus that, together with metacyclic promastigotes, deposit vector saliva into the host skin during the blood feeding process. Sand fly saliva was shown to be an infection enhancer, and counter-intuitively to confer anti-Leishmania protection upon multiple exposures. Yet, most of the works concerning Leishmania and leishmaniasis have a binomial focus (host-parasite) and disregard the contribution of the vector. Such neglect, in the context of anti-Leishmania vaccination is both a limitation, as highlighted by Peters et al that have shown the loss of protection of a supposedly-good vaccine candidate, when tested in the context of vector-transmitted leishmaniasis; and an extravagance, having in consideration that vector saliva is an untapped source of antigens for Leishmania vaccines.

Having in consideration the trinomial nature of this vector-borne disease, we explored in the pre-clinical context, an innovative vaccine approach for human visceral leishmaniasis consisting of three different recombinant proteins (LJL143 from Lutzomyia longipalpis saliva as the vector-derived (VD) component, and KMP11 and LeishF3+, as parasite-derived (PD) antigens), either free, or formulated in Influenza virosomes, and adjuvaned with GLA-SE, a TLR4 agonist. Interestingly, the immune responses generated against the VD protein were reproducibly more robust than those elicited against leishmanial antigens, and were apparently not caused by immunodominance of the PD antigen.

These results directed our focus to the investigation of the influence of an unusual immunization scheme in the memory responses generated by our complex vaccine candidate. Remarkably, priming with the VD protein alone and boosting with the complete vaccine candidate contributed towards an increase of the immune responses to the PD recombinant proteins as well as to total Leishmania antigens (TLA). This prime-boost immunization approach gives relevance to the use of both parasite and vector derived antigens together as an anti-Leishmania vaccine, and contributes to the debate on vaccination in endemic versus non-endemic areas, where people are either constantly or never (respectively) exposed to sand fly bites.

Acknowledgments:

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Correspondence: Email - pedro.cecilio@ibmc.up.pt
SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Stigma: The hidden fiery spear of cutaneous Leishmaniasis in Ethiopia.

M Behailu 1, B Hilde 2, P V Jean 3, V G Johan 4

1 Arba Minch University, Department of Public Health, College of Medicine and Health Sciences, Ethiopia; 2 Department of Primary and Interdisciplinary Care, Faculty of Medicine and Health Science, University of Antwerp-Belgium; 3 Faculty of Medicine and Health Sciences-Epidemiology and Biostatistics Unit Institute of global Health University of Antwerp, Belgium; 4 Institute of Tropical Medicine, Antwerp, Belgium.

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ABSTRACT

Background: Ochollo, a small village is an endemic focus for Leishmania aethiopica infection since its recognition of 1913.

The objective of the study was, to assess what psychological impacts the disease had put forth on people who have the active lesion or has the permanent scars the disease.

The Gap: At least until today, no studies done in Ethiopia to understand the psycho-social impact of the CL scar and/or ulcer.

The Hook: Being the first research to bring the issue of CL associated stigma on board the finding will help unveiling it for a better understanding of the psychological consequences it puts forth.

Results: Five main themes : Fear, Shame, Isolation, Stigma, and Rejection. Change in behavior started after individual contracted CL. Stigma: is the most common and repeatedly expressed form of psychosocial impact on people who are having or had CL. A father of a 12 year old 4th grade student from TK said, “When the lesion holds pus, then the discrimination occurs......”. At home when they sit for dining “…..give her own with another separate plate... or they say to her to cover her face ....In school and community same...... Another 48 years old male participant from KA saying, “My eyes were affected. One day while talking with a person he directly insulted me saying ‘You man with the Bolbo’. After that, I was so ashamed, and psychologically traumatized and then I wanted even to remove the scar from my face by peeling off the scar from my face so that nobody insults me”.

Conclusion and recommendation: Although, some CL lesions heal spontaneously leaving life-long scars, the majority of lesions are non-healing or chronic with sequels leading to disfigurement or mutilation with severe social and economic consequences. The results indicate that Cutaneous leishmaniasis put forth a psychological impact up on individuals who contracted the disease and having a lesion is attached to stigma.

Acknowledgments:
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References:

Correspondence: Email - jyangriensven@itg.be
Cloning, expression and production of rK39 from Iranian strain of Leishmania infantum for serodiagnosis of visceral leishmaniasis in human

Bibi Razieh Hosseini Farash 1, 2, 3, Mehdi Mohebali 3, Abdolmajid Fata 1, 2, Seyed Aliakbar shamsian 3, Bahram Kazemi 4, Homa Hajjaran 3, Behnaz Akhoundsi 3, Reza Raofian 2, 3.

1 Cutaneous leishmaniasis Research Center, School of Medicine, Mashhad, Iran; 2 Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; 3 School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; 4 Faculty of Medicine, Shaheed Behesht University of Medical Sciences, Tehran, Iran.

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Abstract

Introduction: Visceral leishmaniasis (VL) caused by Leishmania infantum is the most severe form of leishmaniasis in Iran [1]. A high mortality rate has been reported without accurate diagnosis and treatment of VL [2]. This study aimed to investigate on gene cloning and preparation of K39 immunodominant antigen of Iranian strain of L. infantum and its evaluation on diagnosis of VL in Iran.

Material and Methods: The band of interest of k39 was ligated into pCR 2.1-TOPO and pET-32a (+), respectively after PCR amplification. The sequences of recombinant plasmids were analyzed. Protein expression and purification of rk39 were performed and along with, the whole cell and DAT antigens were prepared to compare the results with recombinant protein. For the final step, 84 positive and 86 negative serum samples of human for VL, are being collected to evaluate sensitivity and specificity of new recombinant antigen for serodiagnosis of VL.

Results: The sensitivity of rK39-ELISA has been reported 85.7% with specificity of 86% for diagnosis of Human VL (HVL) (≤1:800) while showed 100% sensitivity in symptomatic people (≤1:3200). The kappa index was calculated for this recombinant antigen to find the level of agreement with DAT. This value was 0.718 and has an excellent agreement with gold standard.

Conclusions: On the whole, the successful rk39 cloning and new recombinant antigen which have performed in this study could help to serodiagnosis of HVL especially in where L. infantum is the main causative agent. Furthermore, this would make this possibility to discuss about the real sensitivity of rk39 prepared by Iranian L. infantum and its application along rk26 protein to diagnosis VL in Iran.

Keywords: Visceral leishmaniasis, Leishmania infantum, K39 immunodominant antigen, human

References:

Correspondence: Email - hoseinfr@ums.ac.ir
A double-sided perspective of leishmaniasis: the lab vs the field experiences

Carla Maia*, José Manuel Cristóvão, Sofia Cortes, Maria Odete Afonso, Ricardo Parreira, Lenea Campino

Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal.

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Abstract

Leishmaniasis caused by the protozoan Leishmania infantum and transmitted by the phlebotomine sand flies Phlebotomus perniciosus and P. ariasi is an endemic zoonosis in Portugal [1]. Dogs are the major host for these parasites, and the main reservoir host for human infection.

The aim of this communication is to update the research on Leishmania infection in cats and vectors and in vitro drug susceptibility performed by Leishmaniases group of the Institute of Hygiene and Tropical Medicine, University Nova of Lisbon in the last years. L. infantum infection in cats has been evaluated in southern Portugal [2-4]. In addition, several phlebotominae surveys were carried out and vector species were found infected with L. infantum. DNA of L. major and Leishmania spp. has been detected in Sergentomyia minuta specimens [5-9]. Regarding in vitro studies, strains isolated from dogs showed low susceptibility to the drugs used in canine leishmaniasis therapy and artemisinin-derived trioxanes and nonclassic metallointercalators with dipyrudophenazine seem to be good candidates for further studies in the context of leishmanial treatment [10-12]. Data reveal that Portugal is a hypoendemic country for human leishmaniases but the prevalence rate of canine and feline infections is a concern for the control of this zoonosis. The on-going and recurrent detection of leishmaniasis in dogs, cats, other mammals and vectors reinforces the importance of surveillance with systematic epidemiologic surveys. Increase awareness of the veterinary community, owners and public health authorities regarding this zoonosis is also warranted.

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References:

Correspondence: Email - CarlaMaia@ihmt.unl.pt
Arginine metabolism and tumor necrosis factor in leishmaniasis: basic research and clinical aspects

Christian Bogdan 1,2,*

1 Institute of Clinical Microbiology, Immunology and Hygiene, Friedrich-Alexander-University (FAU) Erlangen-Nürnberg and University Hospital Erlangen; 2 Medical Immunology Campus Erlangen, FAU-Erlangen-Nürnberg

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Abstract

Leishmaniasis comprises a spectrum of vector-borne chronic cutaneous, mucocutaneous or visceral diseases that are caused by different species of the protozoan parasite Leishmania. Experimental mouse models have been extremely informative to study and understand the immunopathogenesis of leishmaniasis. Nitric oxide generated by inducible nitric oxide synthase (iNOS or NOS2) following activation of macrophages by interferon (IFN)-γ and tumor necrosis factor (TNF) was shown to be essential for the control of acute and latent cutaneous leishmaniasis elicited by Leishmania (L.) major due to direct anti-leishmanial and immunoregulatory effects. Recently, we found that TNF not only contributes to the induction of iNOS, but also suppresses the expression of the competing enzyme arginase (Arg) 1 in vitro and in vivo by an epigenetic mechanism, thereby helping to maintain the output of NO. In the lecture I will discuss whether Arg1 and/or Arg2 are required for (a) the healing of acute L. major infections, (b) the long-term persistence of L. major parasites, and (c) the progression of non-healing cutaneous leishmaniasis caused by L. mexicana. The clinical implications of this work will also be outlined.

Correspondence: Email - christian.bogdan@uk-erlangen.de
Prognosis in dogs with Leishmaniasis in the Netherlands

Christine Piek 1*, Marja de Jong 1, Aukje Rappoldt 1

1 Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

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ABSTRACT

In recent years the import of foreign stray dogs in the Netherlands has steadily increased from around 8,000 in 2012 to 11,310 dogs per year in 2015. This last number is with 7.5% a considerable part of the total yearly increase of dogs in the Netherlands in 2015. Leishmaniasis is not an endemic disease in the Netherlands. As a direct consequence the Dutch veterinarian has to cope increasingly with the challenge of diagnosis and treatment of Leishmaniasis in dogs. The question is if the progression of clinical disease, response to treatment, and survival in these dogs is comparable to the course of the disease in geographic areas where Leishmania infections are endemic. As a preliminary answer to this question a retrospective study was set up with the aim to determine survival time, response to therapy, and the factors determining prognosis in a cohort of 47 dogs diagnosed with Leishmaniasis. The overall estimated Kaplan-Meier median survival time was 6.4 years (95% CI 1.8-11 years). Univariate Cox regression analysis identified protein-losing renal disease as a strong negative predictor of survival. Multivariate Cox regression analysis identified decrease in haematocrit, and increases in plasma creatinine and serum total protein concentrations as the major factors determining prognosis. Interestingly, survival in dogs classified based on the severity of clinical disease (Canine Leishmania Working Group) using the major criteria proteinuria and azotemia (IRIS stage 3-4 kidney disease, CLWG stage D) as distinguishing features was not significantly different [1,2].

Figure 1 | Estimated survival in Kaplan-Meier survival curves for group A (red line) and B (black line). The dotted lines represent the corresponding 95% confidence intervals

References:

Correspondence: Email - C.J.Piek@uu.nl
Clinical and laboratory features in human visceral leishmaniasis, northeastern Iran, 1997-2017

Elham Moghaddas 1*, Seyed Aliakbar Shamsian 1, Abdolmajid Fata 1, Fatemeh Sadabadi 1, Mehdi Zarean 1, Zahra Navi 1

1 Department of Parasitology and Mycology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

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ABSTRACT

Because of VL happens more in pediatrics at risk of death, accurate detection in an early stage of infection is necessary [1]. The aim of this study resolution of prevalence, clinical and laboratory findings visceral leishmaniasis patients compared to the healthy group. In this retrospective study, 25 confirmed hospitalized VL patients with 15 the healthy children were included between 1997-2017 in Emam Reza hospital, Mashhad, Khorasan Province, Northeastern Iran. Gender, age, WBC, RBC, HGB, ESR, Hct, PT, PLT, AST, ALT, Na, K, BUN and RR were analyzed and compared to the healthy group by SPSS Ver. 20. During 20 years, twenty-five patients were recorded with a gender ratio of two males to one female. All cases presented with hepatosplenomegaly. Fever and RR in the patient group were statistically significant compared with the healthy group. Mean age of the patients was 3.7±4 years. No significant differences (p>0.05) were observed in ALT, K, PT between VL patients and control group. Na, HGB, PLT, and WBC were found the highly significant difference between case and control groups. The number of cases per year markedly decreased from 7.4 cases/year in the 1982-1996, to 1.2 cases/year in the 1997-2017. ALT, K, PT weren’t reliable laboratory parameters in the diagnosis of VL in this study.

Acknowledgments:
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References:

Correspondence: Email - Moghaddase@mums.ac.ir
Nature inspired new compounds against Leishmaniosis, from *Eremurus persicus*

Elisa Vignoni¹, Valeria Cavalloro¹, Serena Della Volpe¹, K M Ahmed², Marisa Machado³, Maria Do Céu Sousa⁴, Emanuela Martino⁵, Simona Collina⁶.

¹ Department of Drug Sciences, University of Pavia, Via Taramelli, 12, 27100 Pavia, Italy; ² Department of Science-Chemistry, University of Garmian, Kalar, Kurdistan Region, Iraq; ³ CESP, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, 4585-116 Gandra PRD, Portugal; ⁴ Faculty of Pharmacy, University of Coimbra, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal; ⁵ Earth and Environmental Sciences Department, University of Pavia, Via S. Epifanio 14, 27100 Pavia, Italy.

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**Abstract**

To date, a limited number of drug candidates are available for fighting *Leishmania*, and there is an urgent need for efficient medical treatments. Within a wider research project aimed at discovering new bioactive compounds from plant kingdom, we focused on *Eremurus persicus* roots. As a result of our investigation, we isolated (R)-Aloesaponol III 8-methyl ether (R)-ASME, showing a remarkable antiprotozoal effect against *L. infantum* with an IC50 of 73 µg/mL and not significant toxicity in a macrophage cell line. The potential of such compound against *Leishmania* infections will lead us to get further insight into the mechanisms of actions studying its behavior in cells.[1]

Basing on the obtained results, we can state that (R)-ASME is a interesting *hit compound* and it constituted the starting point of the herein presented work. Of note, the hit is poorly soluble in water and in aqueous buffers, as a consequence the biological assays are difficult to perform and the results difficult to compare. Accordingly, we designed new water-soluble (R)-ASME-derivatives. To improve the hit physicochemical properties, different molecular modification strategies of (R)-ASME have been planned, taking into account its structural features and molecular reactivity. The investigated approaches consisted in the conjugation of (R)-ASME with either amino acid (AA), since it is considered a useful method for increase compound water solubility [2] or with an hydrophilic moiety. The latter approach allowed us to obtain a stable and water soluble salt. The *in vivo* assays of this ASME-derivative are still in progress.

Moreover, to improve the cellular uptake, and following a drug targeting approach [3], ongoing efforts will be addressed to prepare a biotin-conjugated (R)-ASME derivative. Results will be presented in due course.

**Figure 1** | Molecular modification strategies of (R)-ASME.

**Correspondence:** Email - simona.collina@unipv.it
References:
Leishmaniosis caused by *Leishmania tropica* and *Leishmania major* in dogs

Gad Baneth 1*, Yaarit Nachum-Biala 1

1 Koret School of Veterinary Medicine, The Hebrew University, P.O. Box 12, Rehovot 7610001, Israel.

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**Abstract**

The main cause of canine leishmaniosis worldwide is *Leishmania infantum*. Clinical disease and infection associated with *Leishmania tropica* and *Leishmania major*, two common agents of human cutaneous leishmaniasis in the Middle East and North Africa, have rarely been reported in dogs. This study reports cases of canine infection with *L. tropica* and *L. major* with clinical manifestations in Israel and compares them to dogs with these infections reported from other countries and canine *L. infantum* infection. The identity of the infecting agent was determined by PCR and sequencing. Comparative serology by ELISA was performed using crude promastigote antigen of *L. tropica*, *L. major* and *L. infantum*. Skin lesions in *L. major* infected dogs were ulcerative and located on the muzzle, feet and foot pads and not associated with generalized lymphadenomegaly and splenomegaly. In *L. tropica* infection, skin lesions were proliferative mucocutaneous in young dogs, or associated with widespread dermatitis, lymphadenomegaly and splenomegaly in older dogs with similarity to *L. infantum* infection. ELISA serology with the different whole promastigote antigens separately was not distinctive between *L. infantum*, *L. major* and *L. tropica* canine infections. In summary, *L. tropica* and *L. major* cause clinical disease in dogs. Dogs suspected of leishmaniosis in areas endemic for human infection with these species and with *L. infantum* should be tested by PCR with DNA sequencing to detect infection and discriminate between the three species. The possible role of canines as reservoirs for *Leishmania* species other than *L. infantum* should be further studied.

**Correspondence:** Email - gad.baneth@mail.huji.ac.il
Conventional antileishmanial drug associated with membrane transport modulators represents a new strategy to leishmaniasis treatment

M. Rafael-Fernandes 1, A. Armada 1, G. Alexandre-Pires 2, A. Valério-Bolas 1, A. Rodrigues 1, M. Viveiros 1, G. Santos-Gomes 1

1 Global Health and Tropical Medicine, GHTM, Instituto de Higiene e Medicina Tropical, IHMT, Universidade Nova de Lisboa, UNL, Lisboa, Portugal; 2 CIISA, Centre for Interdisciplinary Research in Animal Health. Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

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Abstract

Leishmaniasis is among the parasitic diseases with the greatest impact in the world. It is considered a neglected disease that affects mainly the poorest populations with high morbidity. Furthermore, the few conventional drugs available for leishmaniasis treatment have high toxicity and limited efficacy. Thus, the development of new drugs or new treatment strategies is urgently needed. The efflux pump activity of ABC (ATP-binding cassette) transporters present on Leishmania-host cell membrane and on parasite may influence the internalization and permanence of drugs within the cells, conditioning the therapeutic activity and favoring parasite survival. Therefore, the present study aims to analyze the efficacy of miltefosine in combination with efflux pumps modulators (EPMs) in reducing intracellular infection. Mouse macrophages infected with Leishmania parasites causing zoonotic visceral leishmaniasis and American cutaneous leishmaniasis were treated with miltefosine in association with EPMs and analyzed for parasite viability. Although EPMs effect differs between Leishmania spp., the association of verapamil (VER), sodium orthovanadate and phe-arg β-naphthylamide (PAβN) with miltefosine seems to restrain drug efflux. VER, which modulates ABCB1 pump efflux and PAβN that modifies the activity of AcrAB transporters establish a synergic relation with miltefosine. The use of EPMs in a combined therapy should be strongly considered, since they may potentiate drug activity, reducing the drug concentration required to inactivate the parasite and, consequently diminish toxic effects and prevent the appearance of resistance. EPM-antileishmanial drug combined treatment can constitute an alternative therapeutic strategy to leishmaniasis conventional treatment.

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Correspondence: Email - gpires@fmv.utl.pt
Molecular mass-screening for vector research on leishmaniasis

Hirotomo Kato1*, Abraham G. Cáceres2, Eduardo A. Gomez3, Yoshihisa Hashiguchi3

1 Division of Medical Zoology, Jichi Medical University, Japan; 2 Laboratorio de Entomología, Instituto Nacional de Salud, Peru; 3 Departamento de Parasitología y Medicina Tropical, Facultad de Ciencias Medicas, Universidad Catolica de Santiago de Guayaquil, Ecuador.

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Abstract

Surveillance of circulating sand fly species in endemic and surrounding areas and identification of sand flies responsible for transmission of *Leishmania* is important for predicting the risk and expansion of the disease. A molecular mass screening that can detect natural infection of *Leishmania* in hundreds of sand flies with minimum effort was established (Fig.1) [1]. Sand fly species were simultaneously identified by PCR-RFLP of the18S rRNA gene using same DNA extract as a template (Fig.2) [2,3]. This method was applied to field research in Ecuadorian and Peruvian Andes, and 3 of 192 and 1 of 462 sand flies, respectively, were positive for *Leishmania* minicircle DNA. In these areas, Lutzomyia ayacunchensis and Lu. peruvensis were identified as vectors of *Leishmania* (*Leishmania*) *mexicana* in Ecuador and *L. (Viannia) peruviana* in Peru, respectively [4], corresponding to previous reports. Further, the natural infection of sand flies by *Leishmania* was examined in the Department of Huanuco of Peru, in which cutaneous leishmaniasis caused by a hybrid of *L. (V.) braziliensis*/*L. (V.) peruviana* is endemic and the vector is not known. A total of 2,997 female sand flies were analyzed, and a hybrid of *L. (V.) braziliensis*/*L. (V.) peruviana* was detected from one *Lu. tejadae*, strongly suggesting that *Lu. tejadae* is responsible for the transmission of the hybrid *Leishmania* circulating in this area [5]. In addition, loop-mediated isothermal amplification (LAMP), which can amplify target gene within 1 hour under isothermal conditions, was adopted as an alternative to PCR for mass-screening of sand flies [6]. Since these methods can process a large number of samples with minimum effort, they will be powerful tools for vector research on leishmaniasis.

![Figure 1](image1.png) Detection of *Leishmania* DNA within sand flies

![Figure 2](image2.png) Genotyping of sand flies

Correspondence: Email - hirok@jichi.ac.jp
References:
NOD2 pathway implication in *Leishmania tropica* infection

I Varotto-Bocazzi 1, D Scaccabarozzi 2, S Villani 3, S Zava 2, I Cavicchini 3, R Nodari 1, S Delbue 3, I Colombo 2, S Epis 1, N Basilico 3, Y Corbett 1,2

1 Department of Biosciences and Pediatric Clinical Research Center, University of Milan, Italy; 2 Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy; 3 Department of Biomedical, Surgical and Dental Sciences, University of Milan, Italy.

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**Abstract**

*Leishmania tropica* is the etiological agent of cutaneous leishmaniasis. Pattern recognition receptors such as toll-like receptors or nucleotide oligomerization domain-like receptors (NLR) have been associated with the disease. The role of NOD2, a member of the NLR family, in innate immune responses against *L. tropica* has been investigated.

Interferon gamma (IFN-γ)-primed or unprimed immortalized mouse bone marrow macrophages (BMDM)-wild type (WT) or NOD2−/− were infected with *L. tropica* at different parasite/macrophage ratios (i.e. 2.5, 5, or 10:1), for 24h. Controls, such as medium alone, muramyl dipeptide (MDP), or lipopolysaccharide (LPS), were included. Levels of cytokines or nitrite released into supernatants were measured through ELISA or Griess reagents. Levels of inducible nitric oxide synthase (iNOS) mRNA and protein were obtained through Real-Time PCR and Western blot analyses, respectively. Also, unprimed BMDM-RIP2−/− or CARD9−/−, two downstream components of NOD2 activation, were stimulated with the same ratios of *L. tropica* or controls, and the production of the pro-inflammatory cytokine TNF-α was compared with BMDM-WT.

Data showed that *L. tropica* did not induce the production of nitric oxide in unprimed BMDM-WT. *L. tropica* induced higher levels of nitric oxide in IFN-γ-primed BMDM-WT than in unstimulated cells. Stimulation due to *L. tropica*, or the control MDP (known to activate NOD2), was abrogated in the BMDM-NOD2−/−, but not LPS, as so the expression levels of inducible nitric oxide synthase (iNOS) mRNA or protein. In addition, NOD2, RIP2 or CARD9 showed to be involved in the induction of TNF-α release from BMDM. These data suggest an involvement of NOD2 pathway in innate immune response to *L. tropica* infection.

**Correspondence:** Email - ilaria.varotto@unimi.it
A new epitope-based peptide vaccine against human leishmaniasis

Joana Pissarra 1*, Philippe Holzmuller 2, Rachel Bras-Gonçałves 1, Amel Garnaoui 3, Jean-Loup Lemesre 1.

1 UMR 177 INTERTRYP IRD, CIRAD, Univ. Montpellier (I-MUSE), Montpellier, France; 2 UMR CMAEE, Centre de coopération International en Recherche Agronomique pour le Développement (CIRAD), Montpellier, France; 3 Laboratoire d’Immunologie, Vaccinologie et Génétique Moléculaire, Institut Pasteur de Tunis (IPT), Tunis, Tunisia.

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Abstract

Vaccine development against leishmaniasis is supported by evidence of natural immunity against infection, mediated by a dominant Th1 response and production of IFN-γ, IL-2 and TNF-α by polyfunctional CD4+ and CD8+ T cells, ultimately leading to macrophage activation and parasite killing. The discovery of immunodominant epitopes responsible for natural protection remains a challenge and a hurdle to vaccine development.

Excreted-secreted proteins are important virulence factors present throughout Leishmania life stages and induce durable protection against infection in dogs. Our rationale for the development of a human vaccine is to identify the immunodominant epitopes present in the Leishmania secretome, and design and synthesize peptides able to induce robust and durable immunity against all major parasitic stages and across the most clinically relevant species of Leishmania.

The secretome of 6 main pathogenic species was identified by Mass Spectrometry and conserved candidate antigens were searched. We selected a total of 52 protein candidates – corresponding to 28 proteins previously described as vaccine antigen candidates, and 24 new antigen candidates through a reverse vaccinology approach. The selected proteins underwent in silico HLA-I and –II epitope binding prediction analysis, with world coverage regarding HLA restriction. Strong binders were selected through an automated R script developed in-house, according to strict criteria. The automated script allowed us to greatly restrict the epitope list, and ultimately select 50 HLA-I and 24 HLA-II epitopes, synthesized as peptides.

Experimental validation of selected peptides is ongoing, wherein we aim to demonstrate their ability to induce Th1 and cytotoxic responses in human cells, from different immunity status (healed, asymptomatic and naïve). To test pre-existent Leishmania-specific memory responses, total PBMC assays are performed to detect IFN-γ production and other-Th1 associated markers. To test the naïve repertoire for specific precursors, T-cell co-culture assays are performed, with several rounds of cellular amplification due to the rarity of specific cells, and search for specific IFN-γ production by ELISpot. Promising peptide candidates will proceed to multi-epitope peptide design, which will be further tested and included in a final vaccine formulation.

Through the combination of proteomic analysis and in silico tools we were able to swiftly identify promising antigen candidates. We further established the secretome as an optimal starting point for vaccine development and the proposed in silico pipeline provides a rapid selection of the best epitopes, with great immunogenic potential. Validation of candidate peptides is performed exclusively with human samples, enabling conclusive immunogenicity testing while avoiding high-cost animal trials with limited extrapolation for human immunity. Together, the proposed strategy will provide us a very strong base for a vaccine formulation against human leishmaniasis and allow to fast-track translation to the field.

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Correspondence: Email - joana.pissarra@ird.fr
Leishmanicidal activity of 7-amino-1,2,4-triazolo[1,5-a]pyrimidine Cu(II) complexes

J M Méndez-Arriaga 1,2*, I Oyarzabal 3, G Escolano 4, A Rodríguez-Diéguez 1, M Sánchez-Moreno 2, J M Salas 1


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ABSTRACT

Looking for the design of new drug candidates for potent, selective and less toxic leishmanicidal therapeutic agents, our research group has been working with triazolopyrimidine derivatives to form new antiparasitic metal complexes during last decades [1]. Two triazolopyrimidine complexes have been obtained from reaction between 7-amino-1,2,4-triazolo[1,5-a]pyrimidine (7atp) and Cu(II) salts. Crystal structures of [Cu₅(μ-7atp)₄Cl]Cl₂·4H₂O (1) and [Cu₇(μ-7atp)₄(H₂O)₃(NO₃)₃]Cl·H₂O (2) have been studied by X-ray diffraction methods and characterized by spectroscopic and thermal analysis. Magnetic studies of these dinuclear complexes have revealed the existence of moderate antiferromagnetic interactions between the copper ions, with J values of -91.2 and -96.1 cm⁻¹ respectively. The antiparasitic activity of these new complexes has been studied in vitro against three different strains of Leishmania spp. and Trypanosoma cruzi, showing a higher efficacy than the 7atp ligand and the reference commercial drugs Glucantime and Benznidazole [2].

Figure 1 | Perspective view of complexes 1-2

![Perspective view of complexes 1-2](image)

Figure 1 | Comparative SI values between reference drugs, pure 7atp ligand and complexes.

![Comparative SI values between reference drugs, pure 7atp ligand and complexes](image)

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References:

Correspondence: Email - qnendez@ugr.es
Characterization of a new focus of visceral leishmaniasis: canine, human and vector components

Joziana Muniz de Paiva Barçante 1, Sidney Ferreira 1, Thiago Pasqua Narciso 2, Richardson Costa Carvalho 3, Ingrid Marciano Alvarenga 2, Alexandra Almeida Pinheiro Chagas 3, Marina Martins Oliveira 2, Thales Augusto Barçante 1

1 Health Science Department, Federal University of Lavras; 2 Veterinary Medicine Department, Federal University of Lavras

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ABSTRACT

Visceral leishmaniasis (VL), or Kalazar, is a vector-borne disease that continues to increase although the classical control measures have been implemented. VL presents high lethality in the cases not treated properly and has spread around the world. This neglected disease is underestimated since it is unnotified in many endemic countries. With the emergence of foci of the disease in urban areas, VL represents a significant burden in public health in Brazil, which is among the six countries that harbor over 90% of the cases worldwide [1,2]. All regions of this country are related to the presence of VL. In the State of Minas Gerais (MG), the disease has spread dramatically in the last 59 years. In this scenario, human and canine cases and infected vectors have been registered to suggest a clear expansion of disease. Therefore, it is essential that health professionals be familiar with the clinical patterns of VL to proceed with the correct diagnosis and proper treatment soon [3,4]. Considering that canine cases usually preceds human VL [5-7], the early detection of new geographical areas with canine VL cases is a critical point for starting or improving the epidemiological surveillance of leishmaniasis. Since the first confirmed case of canine VL occurred in 2013 and the first report of Lutzomyia longipalpis, in 2015 in the municipality of Lavras, health education actions have been implemented by the authors. In January 2017, the first autochthonous case of human VL was diagnosed, which elevated to 226 the number of municipalities with reported cases of VL in the State of MG. As health education is considered an essential resource to control leishmaniasis, the educational actions have been applied to the health professionals, teachers of elementary education and high schools and the broad community by the distribution of informative brochures, flipcharts, and interactive lectures. After this first report of human VL, other five cases were diagnosed in the city of Lavras in 2017 including adults, children, and an elderly man. These reports highlight the need of maintenance of surveillance and control programs including the active search of sandflies, human and canine cases and also public health education. The current situation of Lavras should also be considered as an alert to other near areas where favorable eco-epidemiological conditions of transmission may exist. We believe that the answer to VL challenges is health education and community participation – two activities that do not appear to be high priorities in the majority of endemic areas. The understanding and active participation of the community is pivotal to get success in the control of the disease. With long-term political support, adequate funding and continuous educational programs, we can be able to control VL and virtually any neglected disease. In summary, health education is a precious key to solve our social problems.

References:

Correspondence: Email - joziana@dsa.ufla.br
Evolutionary relationships among protein deacetylases of *Leishmania* and other parasites

Laila Alves Nahum 1-2*, Larissa Lopes Silva Scholte 3

1 René Rachou Institute, Oswaldo Cruz Foundation - FIOCRUZ, MG 30190-009, Brazil; 2 Promove College of Technology, Belo Horizonte, MG 30140-061, Brazil; 3 Oswaldo Cruz Foundation - FIOCRUZ, RJ 21040-361, Brazil.

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**Abstract**

Histone modifying enzymes are potential drug candidates against neglected diseases. They are involved in the regulation of chromatin modifications, thus globally regulating gene expression. Moreover, aberrant epigenetic states are often associated with human diseases, leading to great interest in these enzymes as therapeutic targets. We have analyzed two families of protein lysine deacetylases (HDACs and sirtuins) of humans, *Leishmania* (*L. braziliensis*, *L. donovani*, *L. major*, and *L. mexicana*), *Trypanosoma* (*T. brucei* and *T. cruzi*), *Schistosoma* (*S. japonicum*, *S. haematobium*, and *S. mansoni*), and *Plasmodium falciparum*. Potential homologues in the predicted proteomes of these taxa were identified by using hidden Markov model profiles. We reconstructed the evolutionary relationships of protein sequences by Bayesian inference and maximum likelihood method. Our results showed that parasite proteomes have diverse protein deacetylases (HDACs and sirtuins) and the evolutionary relationships among them are well supported. Experimental data described elsewhere suggests these enzymes as common drug targets among parasites. Our work has improved the functional annotation of approximately 63% HDACs and 51% sirtuins in the selected taxa providing insights for future experimental characterization. Together, our work contributes to a better understanding of parasite proteins and might support the development of new inhibitors and strategies against human diseases.

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**References:**


**Correspondence:** Email - laila@minas.fiocruz.br
Levels of trace elements in sérum of dogs and their correlation to occurrence of leishmaniasis

Raffaele Corso 1*, Mauro Esposito 2, Giuseppe Picazio 2, Pasquale Gallo 2, Laura Manna 1

1 Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli; 2 Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici.

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Abstract

The chemical contamination of the environment is a considerably serious problem. The majority of dangerous chemical pollutants, considered particularly harmful for humans, especially children [1], are heavy metals, relating to water and soil contamination [1-3]. The main threats to human health are associated with exposure to lead, cadmium, mercury and arsenic. Although adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues and is even increasing in some areas [2]. The World Health Organization (WHO) estimates that about a quarter of all diseases are due to prolonged exposure to environmental pollution. Certain heavy metals have been reported to seriously affect the immune system potentially resulting in a broad range of harmful health effects. The link between metals and immune function has been studied for many years; developmental exposure to lead results in persistent immune alterations in rodents, including reduced antibody levels, altered cytokine production [4]. Some studies were conducted on the heavy metal content in sérum of dogs to evaluate the degree of exposure in urban or industrial areas [5]. Another study checked a potential link among histopathology and some trace elements in canine visceral leishmaniasis, a severe and fatal systemic chronic inflammatory disease [6]. The present study was aimed at determining trace element concentration in sérum of dogs to evaluated if high levels of heavy metals are a factor contributing to vulnerability to leishmaniasis. Blood samples were collected from 19 leishmanioatic dogs and 74 not leishmanioatic dogs. All the 93 animals were from different geographic areas of Campania Region, endemic for L. infantum.

The analysis was carried out using a validated analytical method based on inductively coupled plasma massspectrometry (ICP-MS), and the data recorded were statistically processed in order to give a contribution to risk assessment. Blood samples obtained from dogs were kept at room temperature for 30 min and centrifuged at 3000 rpm for 15 min to separate the sérum. The sérum samples were transferred in eppendorf tubes and stored at −80°C until analysis. Aliquots (500 µL) of sérum samples were transferred in metal- free polyethylene tube and they were diluted to 10.0 mL with HNO3 1% (v/v).

The determination of 16 trace elements (As, Hg, Pb, Cd, As, Sr, V, Ni, Se, Cr, Mo, Li, Cu, Zn Mn and Fe) was carried out by an ICP-MS mod NexION 350X (Perkin Elmer, Waltham, MA-USA). All measurements were conducted in duplicate. Trace element concentrations were calculated by using calibration curves and were expressed as mg/L. The limits of quantification (LOQ) were calculated as the blank signal plus ten times its standard deviation, respectively. Monitoring of trace elements was carried out. Regarding the differences between not leishmanioatic versus leishmanioatic dogs, the results obtained in this preliminary study showed that: the mean quantity of Fe was 4,259 µg/mL ± sd vs 2,763 µg/mL ± sd, Mn was 0,006µg/mL ± sd vs 0,008µg/mL ± sd, Sr was 0,057 µg/mL vs 0,064 µg/mL. The differences between the average quantities of the other metals did not show statistically significant differences. Future studies will be needed to assess the correlation of leishmaniosis with sérum metal concentrations.

References:

Correspondence: Email - raffaelecorso@gmail.com
An overview on Leishmaniases in Portugal: epidemiology, clinical presentations and therapy

Lenea Campino ¹

¹ Instituto de Higiene e Medicina Tropical/ Global Health and Tropical Medicine, Universidade NOVA de Lisboa, Portugal.

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Abstract

In Portugal, human visceral leishmaniasis (HVL) is hypoendemic like in other Western European countries. HVL had a high resurgence in Europe in the 1980s and 1990s due to the emergence of the pandemic HIV infection. In recent years, the incidence of co-infection cases has declined sharply. All the notified co-infection cases were in adult patients. Regarding the clinical cutaneous form (CL), only a few cases are presently diagnosed, due to the benign form of the infection, more often being self-healing. More severe cases of CL are found in patients with immunosuppression such as diabetes.

In opposition, canine leishmaniasis (CanL) prevalence continues to reach high infection rates. Last national serological survey has shown prevalence higher to 15%, in some regions of the interior Centre and South of the country.

In Portugal, treatment of HVL with liposomal amphotericin B has a high success rate being the first line treatment. Concerning CanL, the treatment is very diverse and the results are weak.

Correspondence: Email - campino@ihmt.unl.pt
Guanylate binding proteins 2b and 5 as indicators of inflammation during leishmaniasis

Marie Lipoldová 1,*, Yahya Sohrabi 1, Valeriya Volkova 1, Tatyana Kobets 1, Helena Havelková 1, Imtissal Krayem 1, Martina Slapničková 1, Peter Demant 2.

1 Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic; 2 Roswell Park Cancer Institute, Buffalo, USA

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Abstract

Interferon-induced GTPases (guanylate-binding proteins, GBP) play an important role in inflammasonme activation and mediate innate resistance to many intracellular pathogens [1], but little is known about their role in leishmaniasis. We therefore studied expression of Gbp2b/Gbp1 and Gbp5 mRNA in skin, inguinal lymph nodes, spleen and liver after Leishmania major infection and in uninfected controls. We used two different groups of related mouse strains: BALB/c, STS and CcS-5, CcS-16 and CcS-20 that carry different combinations of BALB/c and STS genomes, and strains O20, C57BL/10 (B10) and B10.O20, Ocb-9 and OcB-43 carrying different combinations of O20 and B10 genomes. The strains were classified on basis of size and number of infection-induced skin lesions as highly susceptible (BALB/c, CcS-16), susceptible (B10.O20), intermediate (CcS-20), and resistant (STS, O20, B10, Ocb-9, OcB-43). All tested strains harbored parasites after infection, although the parasite load in resistant strains is low. Some uninfected strains differed in expression of Gbp2b/Gbp1 and Gbp5, especially of Gbp2b/Gbp1 in skin. Uninfected BALB/c and STS did not differ in their expression, but in CcS-5, CcS-16, and CcS-20, which all carry BALB/c-derived Gbp gene-cluster, expression of Gbp2b/Gbp1 exceeds that of both parents. These data indicate trans-regulation of Gbps-pattern of inheritance which is considered to be caused by trans-regulatory effects of non-linked or distant genes. Infection resulted in approximately 10x upregulation of Gbp2b/Gbp1 and Gbp5 mRNAs in organs of both susceptible and resistant strains, which was most pronounced in skin. CcS-20 expressed higher level of Gbp2b/Gbp1 than both parental strains in skin, whereas CcS-16 expressed higher level of Gbp2b/Gbp1 than both parental strains in skin in liver. This indicates a trans-regulation present in infected mice CcS-16 and CcS-20. Immunostaining of skin of five strains revealed in resistant and intermediate strains STS, CcS-5, O20 and CcS-20 tight co-localization of GBP2b/GBP1 protein with most L. major parasites, whereas in the highly susceptible strain BALB/c most parasites did not associate with GBP2b/GBP1.

Conclusion: Expression of Gbp2b/Gbp1 and Gbp5 was increased even in organs of clinically asymptomatic resistant mice. It suggests a hidden inflammation, which might contribute to control of persisting parasites. This is supported by the co-localization of GBP2b/GBP1 protein and L. major parasites in skin of resistant and intermediate but not highly susceptible mice [2].

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References:

Correspondence: Email - lipoldova@img.cas.cz
Visceral Leishmaniasis Discovered in Northern Somalia

Mikko Aalto*.

* Bosaso General Hospital, Bosaso, Somalia;

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ABSTRACT

Discovering a new disease like Visceral Leishmaniasis (VL) in a geographic area where it is both unknown and unknown to exist is difficult if diagnosing needs special tests which are not available, just because the disease is not known to exist. In the Northern Somalia there had been long time small children with waisting, pancytopenia and splenomegal and ending always fatally. As VL had been unknown to local doctors, these children were "diagnosed" with leukemia, for which no treatment was available any case.

Because no rK39 RDTs were not available, first few suspicions in 2014 were checked by bone marrow biopsy and making Giemsa stained imprints from them. Without special expertise the microscopy for amastigotes proved out to be very difficult and several hours of microscopy was needed to be moderately convinced that "the observed objects" are really amastigotes. On contrary the dramatic and rapid cure with Sodium Stibogluconate (SSG) proved much convincingly the diagnosis – or at least that a good treatment was found. Only after and because of a few children being diagnosed with VL and treated successfully we received from WHO also rK39 RDTs (Kalazar Detect™ InBios International Inc.). However these RDTs seemed to have sensitivity of around 70% and were negative with many children who had very advanced state of VL and who responded well and were cured with SSG. Hence in 2016 all suspected patients had combination of RDT, bone marrow or spleen aspirate Giemsa staining for amastigotes and promastigote culture done. Also Malaria RDTs, HIV tests, chest x-rays, blood cultures etc were used routinely for other pathologies. Direct microscopy results were expressed not as "positive" or negative" but on a scale from 0 to 4 where 0 represents "convincingly negative" and 4 "convincingly positive".

30 patients had bone marrow or spleen aspirations and promastigote cultures done. Most patients have all relevant records for this retrospective analysis. One interruption in the supply of SSG occurred with increased deaths. In other times a dramatic response to the SSG was used as a fourth diagnostic criteria. 23 patients had positive promastigote cultures and 7 were negative. Of 22 promastigote culture positives with RDT records 6 were negative by RDT, giving 73% sensitivity. 29 patients had RDT records, 7 were negative and 22 were positive. Of these 7 RDT negatives 6 were positive by promastigote culture.

With "real world stains, microscopes, specimens and microscopist" all 7 direct microscopies with all 7 RDT negative patients were inconclusive, i.e. not clearly positive or negative for amastigotes.

Conclusion: Promastigote culture with simple house made culture media is feasible in very low resources setting and is very useful with rK39 RDT negative patients. In our hands direct microscopy for amastigotes did not contribute anything for RDT negative patients.

References:

Correspondence: Email - mikko_aalto@yahoo.com
Refugee Migration and Cutaneous Leishmaniasis, in Turkey

Nebiye Yentur Doni1,2,

1 Harran University Vocational School of Health Services Department of Medical Microbiology, Sanliurfa, Turkey; 2 The Ohio State University, Wexner Medical Centre, Department of Pathology and Microbiology, Columbus, Ohio, USA.

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Abstract

Background: By the beginning of 2018, 3,572,565 Syrians had fled Turkey since the civil war began in Syria in 2011, due to open border policy of Turkish government. Of these, 224,334 take shelter in temporary refugee camps, while 3,348,231 remain living outside of refugee camps [1]. War and conflicts cause the re-emergence of infectious diseases including cutaneous leishmaniosis (CL) as a result of collapsed health care infrastructures and population migration. When the refugees migrate to new places they might be exposed to new infections, and introduce and transmit their diseases to new areas and, populations. Cutaneous leishmaniosis is the most common form of the leishmaniosis, responsible for about three-fourths of the total cases globally [2]. Although CL has been well known for many decades in Turkey, an increasing prevalence of CL is seen in both endemic and non-endemic areas in correlation with the Syrian refugee influx. In light of these data we aimed to evaluate the effects of Syrian conflict on CL.

Methods: The data presented in this study are gathered primarily from the reports written after the Syrian civil war began (2011), about the causative agents of cutaneous leishmaniosis among Syrian refugees in Turkey. The Cochrane Library, MEDLINE, Embase, and US National Library of Medicine National Institutes of Health; Web of Science, Web sites of World Health Organization, United Nations Refugee Agency, and the Health Ministry of Turkey were searched with the terms ‘cutaneous leishmaniosis’ ‘Turkey’ ‘Syria’ or ‘Syrian and ‘migration’, and ‘refugee’.

Results: Turkey is an endemic country for CL and south/south eastern regions of Turkey are already hyper endemic regions that CL cases are mostly reported in. However, CL is not limited to these regions owing to increases of Syrian CL cases and permanent or seasonal migrations of CL cases to nonendemic areas. Cutaneous leishmaniosis cases have also been reported from the Central Anatolia and Ege Regions. Leishmania tropica (L. tropica) is the major causative agent of CL; however Leishmania infantum is the causative agent in the eastern Mediterranean region of Turkey [3]. It has been reported that L. tropica is detected among Syrian refugees [4-8] and, non-endemic parasite strains Leishmania major and Leishmania donovani were introduced by incoming refugees [5, 7]. In a study that investigated the dermatological infectious diseases, CL was the most diagnosed among Syrian patients [6].

Conclusions: This study presented once again that, there has been dramatical increase in CL incidence in recent years in Turkey, both in terms of clinical cases and CL parasite strains, which might be related with continuing influx of Syrian refugees. It should be kept in mind that when prevention and control methods are ignored, the considerable influx of CL cases, and the sand flies which transmit CL cases might spread CL and cause an outbreak.

Keywords: Cutaneous leishmaniosis, Syrian refugees, Turkey

References:

Correspondence: Email - n_doni@hotmail.com
Target-based drug design and development of drug delivery systems to tackle leishmaniasis

Nicola Micale۱*, Angela Scala۱ and Anna Piperno۱.

۱ Laboratory of Nanomedicine, Biomaterials and Heterocyclic Compounds - Department of Chemical, Biological, Pharmaceutical and Environmental Sciences - University of Messina, Viale F. Stagno D’Alcontres 31, I-98166 Messina (ITALY)

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ABSTRACT

Leishmaniasis is one of the most neglected, overlooked and deadly vector-borne infectious diseases afflicting primarily people of tropical and sub-tropical areas of the developing countries. Chemotherapy is currently the only effective approach to treat all forms of leishmaniasis. However, its effectiveness is severely limited due to high toxicity, long treatment length, drug-resistance or inadequate mode of administration. An effective vaccine for humans has not been developed yet. This scenario created an urgent and continuous need for new interventions. In our ongoing search in this field, we identified new molecular scaffolds (i.e. decorated fused benzo[d]thiophene [1], enolizable cyclic 5,8 -triketone [2] and 1,2-substituted-1H-benzo[d]imidazole [3]) targeting Leishmania mexicana CPB2.8CTE, one of the more promising targets for antileishmanial drug design. Moreover, we successfully developed new delivery systems based on biocompatible polymeric backbones such as PLGA-PEG copolymer [4] and hyaluronic acid (HA) [4,5] containing the antileishmanial drug pentamidine (Pent). The novel PLGA-PEG-Pent and HA-Pent bioconjugates have been proposed, respectively, as non-targeted and targeted drug delivery systems.

![Figure 1](image)

**Figure 1** | Identified lead structures targeting *L. mexicana* CPB2.8CTE; B) Pentamidine bioconjugates.

Correspondence: Email - nmicale@unime.it
References:
Sand fly salivary proteins and their potential use in the control of canine leishmaniasis

Petr Volf 1*, Tereza Lestinova 1, Tatiana Spitzova 1, Laura Willen 1.
1 Dept. Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

Available Online: 30 December 2018

Abstract

During Leishmania transmission, sand flies inoculate the host with saliva composed of a diverse group of molecules (proteins and peptides) which have anti-hemostatic, anti-inflammatory and immunomodulatory properties (reviewed in [1]). Leishmania parasites benefit from a saliva-altered local immune reaction. In naïve hosts, saliva exacerbates the infection, causing “enhancing effects”, reflected by larger lesions and higher parasite numbers [2]. In contrast, hosts repeatedly exposed to bites of uninfected sand flies or immunized by salivary proteins were usually protected against Leishmania infection [3, 4]. Therefore, sand fly salivary antigens are proposed as vaccine candidates against leishmaniasis.

Hosts repeatedly bitten by sand flies also develop specific anti-saliva antibodies. Levels of anti-saliva IgG reflect the intensity of exposure to sand flies and thus can be used in epidemiological studies, e.g. to measure the effectiveness of vector-control campaigns or as a marker of risk for Leishmania transmission (reviewed in [1]). Around the Mediterranean basin, the sand fly Phlebotomus perniciosus is the principle vector of Leishmania infantum, the causing agent of canine leishmaniasis. A salivary Yellow protein SP03B of P. perniciosus has been shown as a valid biomarker to estimate dog exposure to P. perniciosus [5, 6]. Since standard serological methods are impractical and time-consuming in field conditions, we developed the rSP03B sero-strip, a rapid test that can be immediately applied to screen large cohorts of dogs for the presence of anti-P. perniciosus antibodies. It is highly sensitive and specific and shown to be a valid replacement for standard ELISA assays. It is the first rapid test designed for medical entomology research and it can be employed during epidemiological studies of canine leishmaniasis in the Mediterranean area.

Acknowledgments:
This work was supported by project CePaVIP (CZ.02.1.01/0.0/0.0/16_019/0000759), the Czech Science Foundation (17-10308S) and Research Centre UNCE 204072 and has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 642609.

References:

Correspondence: Email - volf@cesnet.cz
Feral cats as a main reservoir of Leishmania in South of Spain

Sandra López Fernández 1*, Carmen Chicharro 2, Eugenia Carrillo 3, Eduardo Martínez Manzanares 1, Javier Moreno Nuncio 2

1 Universidad de Málaga, Spain; 2 Instituto de Salud Carlos III

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ABSTRACT

The latest studies have showed that domestic cats can be a chief reservoir also, mainly these animals injured by a retroviral diseases like Feline Leukemia (FeLV) or Felines Immunodeficiency (FIV). Both retrovirus proliferate out between cat who lives nearby, in territorial fights, using same drink or feeding points. Based on these studies, it can be expected that outdoor cats which come from settled colonies will show high incidence of leishmanial infantum. At least, it has been hope find animals with antibodies against I. A hundred of feral cats were tested by qualitative serology assay for Leishmaniosis and retroviral diseases (check by IFA, ELISA and PCR).

Seroprevalence in stray cats were 28.5% and it were founded one contradiction between other publications about the comorbidity with retroviral diseases like FeLV or FIV. In this study 14.3% of cats showed antileishmania-antibodies, nevertheless only 3.6% were FeLV positives at the same time than Leishmaniosis and 7.1%were also FIV positives with leishmaniasis. PCR isolated Leishmania DNA in 30.4% of tested cats. Stray cats can play more important fact in leishmaniosis transmission than it were suspected.

Acknowledgments:
Javier Moreno Nuncio, Eduardo Martínez Manzanares and Fernando Fariñas Guerrero, for their invaluable support and help along this years, and believe in my work.

References:

Correspondence: Email - sandralopezfernandez@uma.es
Whole-genome sequencing of Trypanosomatidae clinical isolates reveals *Crithidia*-like species in cases of visceral and cutaneous leishmaniasis in Brazil


1 Federal University of São Carlos, São Carlos, SP, Brazil; 2 University Hospital, Federal University of Sergipe, Aracaju, SE, Brazil; 3 University of São Paulo, Ribeirão Preto, SP, Brazil; 4 National Institute of Allergy and Infectious Diseases, NIAID-NIH, Rockville, MD, USA; 5 Fiocruz- Bi-Institutional Translational Medicine Project, University of São Paulo, Ribeirão Preto, SP, Brazil.

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Abstract

Over the last decades, sporadic reports of coinfections of *Leishmania* with apparently monoxenous trypanosomatids (or monoinfection) have been described (revised by Kaufer et al [1]). The question arises as to whether these unusual infections are occasional findings or are evidence for novel parasites with the potential to threaten public health. Through DNA sequence analysis of parasite strains isolated from patients presenting both visceral (VL) and cutaneous leishmaniasis (CL) symptoms, we identified a new pathogenic trypanosomatid in 33 out of 34 clinical cases diagnosed as VL and in one case of CL in Brazil. The confirmation of a new species was achieved by whole-genome sequencing analyses of seven clinical isolates. By comparing coding sequences of orthologous genes (ranging from 1000 to over 6000 genes) within 36 Trypanosomatidae organisms, we constructed a phylogram based on a total orthologs median matrix (TOMM) and found that the new parasite is closely related to *Crithidia fasciculata*, which parasitizes exclusively mosquitoes and is considered noninfective to humans. Our findings raise concerns about an emerging infectious disease that is easily confused with leishmaniasis, opening a research path to address epidemiological questions about the identification of vectors, reservoirs, and distribution patterns; the reassessment of leishmaniasis cases in Brazil; better methods for parasite species identification; and drug resistance and new treatment options.

Acknowledgments:
This work was supported by grants from the São Paulo Research Foundation–FAPESP, under agreements 2016/20258-0 (Young Investigator Award) and 2013/08216-2 (Center for Research in Inflammatory Diseases), and by CAPES grant number 23038.005304/2011-01 and CNPq grant number 552721/2011-5. SRM received a fellowship from FAPESP (2017/16328-6).

References:

Correspondence: Email - srmaruyama@gmail.com
Sand flies, mycobiota and leishmaniasis

S Epis 1*, E Martin 1, I Varotto Boccazzi 1, L De Marco 2, L Gradoni 3, N Basilico 4, L Sacchi 5, C Bandi 1.

1 University of Milan - Department of Biosciences and Pediatric Clinical Research Center; 2 University of Camerino - School of Biosciences and Veterinary Medicine; 3 Istituto Superiore di Sanità - Unit of Vector-Borne Diseases and International Health; 4 University of Milan - Department of Biomedical Sciences, Surgical and Dental; 5 University of Pavia - Department of Biology and Biotechnology.

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Abstract

The use of yeasts in biological control is limited, probably due to the fact that yeasts are generally regarded as organisms that exert a positive effect on insects, e.g. a nutritional role. However, beyond the nutritional function, there are several other roles that have been suggested for yeasts, but with limited experimental evidences: one of these is the protective or antagonist role of the yeast symbionts against pathogenic microorganisms. Here, we focused our research on the sand fly Phlebotomus perniciosus, the main vector of human and canine leishmaniasis in the Mediterranean area, with the intentions of describing the mycobiota and of investigating if yeasts associated with this insect could exert inhibitory/killing activity against the pathogen Leishmania spp. First, we investigated the mycobiota by culture-dependent approaches (microbiological analyses and the sequencing of the 26S rRNA, ITS rRNA metagenomic analysis, fluorescence in situ hybridisation (FISH) and phylogenomic analysis [1]. Second, we focused our attention on Wickerhamomyces anomalus, an ascomycete yeast well known for its antimicrobial properties; this yeast was phylogenetically characterized and tested against selected yeast strains, proving its killer phenotype [2; 3]. Finally, we tested the in vitro activity of W. anomalus strains against the pathogen Leishmania infantum in order to explore their potential inhibitory/killing property against pathogens. We believe that this study offers the basis for the development of an environmental-friendly and safe “tool” to interfere with sand fly vectorial capacity, which may be included in the integrated approaches for the control of leishmaniasis, a worldwide re-emerging public health problem.

References:

Correspondence: Email - sara.epis@unimi.it
Molecular epidemiology and the investigation of *Leishmania* RNA virus of *Leishmania tropica* strains in Turkey

Muhammed Nalçaci ¹, Mehmet Karakuş², Bahtiyar Yılmaz ¹, Samiye Demir ¹, Yusuf Özbek ³, Seray Töz ³.

¹ Ege University, Institute of Science, Division of Biology, Department of Zoology, Bornova, İzmir, Turkey; ² University of Health Sciences, Department of Biotechnology, Istanbul, Turkey; ³ Ege University, Medical Faculty, Department of Parasitology, Bornova, Izmir, Turkey.

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**Abstract**

In Turkey, cutaneous leishmaniasis (CL) is a major public health threat and causative agent is mainly *Leishmania* (*L.*) *tropica*. We aimed to evaluate the situation of *L. tropica* in Turkey by multilocus microsatellites typing (MLMT) besides *Leishmania* virus (LRV) positivity for better understanding the epidemiology and virulence of the parasite.

*Leishmania* isolates or slide samples obtained from CL patients were analyzed by MLMT. MLMT was performed with 12 highly specific microsatellite markers using genomic DNA. Repeat numbers for each locus were recorded and Delta K (ΔK) calculation, and Bayesian statistics were used for determining the population structure. Delta K calculations were showed three main populations (POP-A, POP-B, POP-C). Further analysis was revealed three subpopulations for POP-B and POP-C, while no subpopulation was identified for POP-A. One of *L. tropica* strain was viscerotrophic, while the rest were dermatropic. Descriptive analysis per populations (K:3) was also performed and POP-B was found to be the most heterogeneous group by having the highest number of alleles (3.00) and *H* ² (0.466) values and *H* ₀ (0.139) values and the mean number of alleles per populations was 2.3.

Additionally, the presence of LRV was investigated in 24 *L. tropica* isolates using agarose gel method after genomic DNA extraction and PCR from cDNA samples after RNA extraction. Positives were sequenced by Sanger method. Seven *L. tropica* strains were found to be positive only in PCR and sequence analyses showed similarities with LRV2.

Our findings revealed that understanding the epidemiological origin of the parasite and virus positivity could be possible using different kind of clinical samples for diagnosis and thus will give a better chance to evaluate the clinical status of the patient by parasitologists and clinicians. Data available here will provide epidemiological knowledge for further studies.

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**Correspondence:** Email: seray.ozensoy.toz@ege.edu.tr
S Synthetic peroxides as potential anti-Leishmania chemotypes

S Cortes 1*, Andrea Mendes 1, Lília Cabral 2, M I S Cristiano 2, I Campino 1

1 Global Health and Tropical Medicine Center, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Rua Junqueira, 100, 1349-008 Lisbon, Portugal; 2 CCMAR and Departamento de Química e Farmácia, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

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Abstract

Leishmania infantum is the causative agent of human and canine leishmaniasis in the Mediterranean basin. The few available drugs show toxic side effects, declining efficacy with increasing drug resistance and are expensive. Artemisinin and its derivatives (ARTs) feature among the most important classes of antimalarial agents. ARTs and related synthetic peroxides have demonstrated high efficacy against Plasmodium sp. and other parasites such as Perkinsus sp. and Toxoplasma gondii [1-3].

Previous results of our team have shown interesting in vitro activity of a small library of peroxides against L. infantum parasites [4]. We are expanding our investigations by evaluating the cytotoxicity and susceptibility of L. infantum and L. donovani promastigotes to an extended collection of peroxides comprising artemisinin-derived trioxanes and synthetic trioxolanes, using anti-Leishmania drugs currently in use as controls. Inhibitory concentrations (IC50s) were calculated and the cytotoxicity of each compound was accessed in the J774A.1 cell line.

Results have shown that the parasites are susceptible to the peroxides tested, at IC50s ranging between 13 μM and 491 μM for both Leishmania species. Moreover, our results have shown that the activity of this chemotype is dependent on the peroxide group. The encouraging results obtained for the activity and safety of trioxolanes, together with their easy access through chemical synthesis, support the relevance of further studies in the context of leishmanial therapy. Susceptibility of the macrophage-amoastigote system and possible modes of action for this group of compounds are being explored.

Acknowledgments:

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References:

Correspondence: Email - scortes@ihmt.unl.pt
An Analysis of Hematological and Biochemical Parameters of cats infected by *Leishmania infantum*

Trícia Maria Ferreira de Sousa Oliveira 1,2*, Diogo Tiago da Silva 2, Maria Luana Alves 2, Julio César Pereira Spada 2, Juliana Cristina Benassi 1, João Augusto Franco Leonel 2, Nuno Wolfgang Pereira 2, Wilma Aparecida Starke-Buzetti 3

1 Departamento de Medicina Veterinária, FZEA, USP, Pirassununga, SP, Brasil; 2 Programa de Pós-graduação em Epidemiologia Experimental Aplicada às Zoonoses, FMVZ, USP, Pirassununga, SP, Brasil; 3 Departamento de Biologia e Zootecnia, UNESP, Ilha Solteira, SP, Brasil

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**Abstract**

In this study we aimed a clinical evaluation and comparative analysis between hematological and biochemical parameters of cats with and without *Leishmania infantum* infection. The cats were separated in two groups: group 1 (G1, n = 5), cats naturally infected by *L. infantum*, diagnosed by parasitological, serological and molecular tests (including sequencing of positive PCR products) group 2 (G2, n = 6) uninfected cats, negative by parasitological, serological and molecular tests. Samples of blood collected directly from the jugular vein were prepared for hematological and biochemical exams, described in Table 1. The mean of each parameter was compared between the experimental groups (G1 and G2) by the t-Student test. In the clinical evaluation, we observed that 100% (5/5) of the cats infected by *L. infantum* presented thinness (body score 1), 80% (4/5) had alopecia and lesions on the body (skin, snout or ear), 40% (2/5) contained hypertrophic lymph nodes and one animal had conjunctivitis. All negative cats were asymptomatic. Regarding hematological examinations we detected a reduction of platelets (p = 0.0062, p < 0.05), increase of erythrocytes (p = 0.0063, p < 0.05) and PPT concentration (p = 4.4832e-06, p < 0.05) in G1 cats compared to G2, whereas leukocytes were increased for G2 cats (p = 0.014, p < 0.05). By the biochemist, the infected cats showed a reduction in the concentration of albumin and aspartate aminotransferase (p = 0.0065 and p = 0.0025, p < 0.05, respectively). The hematological and biochemical data herein described shows an association between *L. infantum* infection and platelet reduction and hyperproteinemia as well as described for canine visceral leishmaniasis. In enzootic areas, significant alterations described, can be suggestive to *L. infantum* infection in cats and specific tests should be recommended.

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**References:**


**Correspondence:** Email - tricia@usp.br
<table>
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<th>Parameters</th>
<th>Reference range</th>
<th>Infected</th>
<th>Non Infected</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red blood cells (10⁶/μL)</td>
<td>5 - 10</td>
<td>8.07 ± 0.51</td>
<td>7.03 ± 0.46</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>24 - 45</td>
<td>33.76 ± 3.21</td>
<td>32.26 ± 2.30</td>
<td>0.3927</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>8 - 15</td>
<td>10.14 ± 0.69</td>
<td>10.36 ± 0.24</td>
<td>0.4708</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>39 - 55</td>
<td>44.32 ± 4.29</td>
<td>46.00 ± 4.49</td>
<td>0.5444</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>30 - 36</td>
<td>30.1 ± 0.87</td>
<td>32.26 ± 2.64</td>
<td>0.1154</td>
</tr>
<tr>
<td>Platelet (10³/μL)</td>
<td>230 - 680</td>
<td>122.4 ± 16.07</td>
<td>265.5 ± 88.25</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>White blood cells (10³/μL)</td>
<td>5.5 - 19</td>
<td>13.78 ± 4.09</td>
<td>21.25 ± 4.03</td>
<td>p &lt; 0.05</td>
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<tr>
<td>Eosinophil (10³/μL)</td>
<td>0 - 1.5</td>
<td>0.98 ± 0.50</td>
<td>1.73 ± 0.22</td>
<td>0.06332</td>
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<td>Neutrophil (10³/μL)</td>
<td>2.5 - 12.5</td>
<td>10.17 ± 3.37</td>
<td>14.15 ± 2.21</td>
<td>p &lt; 0.05</td>
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<tr>
<td>Lymphocyte (10³/μL)</td>
<td>1.5 - 7</td>
<td>2.58 ± 1.04</td>
<td>5.11 ± 2.54</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Monocyte (10³/μL)</td>
<td>0 - 0.85</td>
<td>0.40 ± 0.36</td>
<td>0.25 ± 0.24</td>
<td>0.4293</td>
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<tr>
<td>Urea (mg/dl)</td>
<td>42.8 - 64.2</td>
<td>97.6 ± 16.51</td>
<td>97 ± 14.01</td>
<td>0.9494</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>0.8 - 1.8</td>
<td>1.04 ± 0.21</td>
<td>1.46 ± 0.36</td>
<td>0.05226</td>
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<td>ALP (U/L)</td>
<td>7 - 80</td>
<td>15.78 ± 1.56</td>
<td>13.97 ± 2.50</td>
<td>0.1957</td>
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<td>ALT (U/L)</td>
<td>06 - 83</td>
<td>36 ± 12.94</td>
<td>39.5 ± 20.63</td>
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<tr>
<td>AST (U/L)</td>
<td>26 - 43</td>
<td>21.6 ± 4.97</td>
<td>38 ± 7.56</td>
<td>p &lt; 0.01</td>
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<tr>
<td>Total protein (g/L)</td>
<td>54 - 78</td>
<td>92.6 ± 3.28</td>
<td>72.5 ± 3.50</td>
<td>p &lt; 0.01</td>
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<tr>
<td>Albumin (g/dl)</td>
<td>2.1 - 3.3</td>
<td>1.54 ± 0.11</td>
<td>2.26 ± 0.44</td>
<td>p &lt; 0.01</td>
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</table>
Canine visceral leishmaniasis: serological and molecular diagnosis of dogs infected with *Leishmania infantum* in Brazil

V L R da Matta *, L F S Batista 1, M D Laurenti 1, R M Silva 2, J E Tolezano 2, H R L R de Lucca 2

1 Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (LIM50)- Brasil; 2 Instituto Adolfo Lutz de São Paulo e Rio Claro- Brasil.

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**Abstract**

In countries where visceral leishmaniasis (VL) is a serious public health problem, such as in Brazil, the identification of infected dogs and their succeeding removal from the endemic area is essential to VL control, since dogs are the main reservoir of the zoonotic VL and capable to transmit *Leishmania infantum* to the vector efficiently[1]. Thus, to assure a reliable diagnosis of infected dogs, the Brazilian Ministry of Health recommends the sequential use of two serological tests. Up to December 2011, ELISA was the screening method and the Indirect Immunofluorescence Assay (IFA) the confirmatory one (old protocol). After that, it was introduced a quick assay accomplished in a Dual-Path Platform (DPP) followed by ELISA (new protocol). Herein, we compared the performance of both protocols using serum samples obtained during a cross-sectional investigation in 2010 (n= 186) and in 2012 (n=856) in an endemic area for VL in southeast Brazil. The current protocol estimated lower prevalence rates of canine infection than did the old protocol, both in 2010 (3.8% x 9.7%) and in 2012 (0.5% x 4.4%). The low concordance of ELISA with the positive DPP results contributed to a considerable decay of the final prevalence (Fig 1). We understand that one of the main forces related to this disagreement relies on sera reactivity to different parasite antigens, i.e., with *L. infantum* recombinant fusion protein rK28 (k9+ k39+ k26) of the DPP, and with total soluble proteins of *L. major*-like in the ELISA. The same did not occur between ELISA and IFA (old protocol), both based on *L. major*-like reactivity. Of note is that DPP did not show any cross-reactivity with sera (n=22) of dogs with other pathologies, but ÉLISA partially reacted with sera of dogs with neoplasia, babesiosis, ehrlichiosis, and Chagas disease. In this regard, our previous study with a well-defined population consisting of dogs with proven *Leishmania* infection showed that DPP presented higher specificity (95.1% x 77.8% x 69.1), positive predictive value (95.1% x 81.1% x 76.6%) and positive likelihood value (18.3% x 4.1% x 3.1) in comparison to ELISA and IFA, respectively. Besides, DPP detected both asymptomatic and symptomatic dogs in equal proportions [2]. Alternatively, PCR with non-invasive samples seems to be a good tool for the canine VL diagnosis. In a concomitant study, conjunctival and buccal swabs from infected dogs (n= 58), when processed together, yielded a positivity rate equivalent to lymph node, and above of that found in blood in symptomatic dogs, reinforcing findings of our previous investigation performed in another endemic area for VL [3]. In conclusion, the current Brazilian serological protocol, that now includes the DPP, presents good accuracy, and has potential to misdiagnosis fewer dogs than the previous one, principally by reducing false-positive results. Respective to the molecular diagnosis, the effective detection of *Leishmania* in canine non-invasive samples is quite relevant for the differential VL diagnosis with other diseases that provoke overlapping signs and symptoms.

**Acknowledgments:**

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**References:**


**Correspondence:** Email - mattav@usp.br
**Figure 1** Prevalence of canine infection in endemic area for VL based on two Brazilian serological protocols (ELISA/IFA, DPP/ELISA)
Canine Visceral Leishmaniasis as a fibrotic disease

Wagner Luiz Tafuri 1*, Adriano Francisco Alves 1, Izabela Ferreira Gontijo Amorim 2; Ramon Alencar Pereira 2; Igor Matheus Valadares Mendes Madeira 1; Rodrigo S. Castro Lucélia Coimbra 1; Washington Luiz Tafuri 2‡

1 Departamento de Patologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais; 2 Departamento de Anatomia Patológica, Faculdade de Medicina, Universidade Federal de Minas Gerais; ‡ - in memoriam.

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ABSTRACT

We propose that Canine Visceral Leishmaniasis (CVL) is a systemic fibrotic disease, as evidenced by the wide distribution of fibrosis that we have found in the dogs suffering from chronic condition. The inflammatory cells apparently direct fibrosis formation. We have described an intense collagen deposition (fibropoeise) in livers, spleen, cervical lymph nodes, lung and kidney of all dogs naturally and experimentally infected with Leishmania (L.) infantum. In fact, all infected dogs showed higher numbers of reticular fibers than controls independently of the clinical status (besides fibropoeise seems to be more prominent in symptomatic dogs). Thus, we have investigated some aspects of this question by analyzing the expression pattern of different known fibrosis markers in distinct canine organs for example: (1) in livers positive correlation has been found among fibropoeise, chronic granulomatous reaction, parasite tissue load and expression of smooth muscle alpha-actin (α-SMA) (a superior marker for activated HSC cells than vimentin and cytokeratin); (2) in lungs the main histopathological picture is chronic and diffuse interstitial pneumonitis. The thickened interalveolar septa is characterized by the cellular exudate (mostly plasma cells, macrophages and lymphocytes) associated with collagen deposition type III (reticulin) and I. Myofibroblasts were characterized by immunohistochemistry based on α-SMA, vimentin, cytokeratin, E-cadherin, Snail antigen homolog 1 (SNAI-1), and transforming growth factor-beta (TGF-β). All myofibroblasts markers are higher in naturally infected dogs compared to uninfected dogs; (3) in kidneys animals has been revealed glomerular and interstitial fibropoeise associated with different types of glomerulonephritis and chronic interstitial nephritis. As seen in livers and lungs, myfibroblasts markers as α-SMA and vimentin are notable in both glomerulus and tubulointerstitial fibrosis process. The expression of the cytokine TGF-beta was also higher than uninfected dogs. Thus, we have concluded that mesenchymal cells are active in promoting changes in the extracellular matrix in all studied organs of dogs naturally infected with canine visceral leishmaniasis reporting a systematic fibrotic picture.

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Correspondence: Email - wagnertafuri@gmail.com
**Figure 1** (a-b): Liver sections of uninfected and infected dogs naturally infected with L. (L.) infantum: (a) Control dog – note a delicate network (weak colour black staining) of intralobular hepatic reticular fibres (arrows). (b) Symptomatic dog – note conspicuous collagen thickening in the space of Disse (stronger black staining of intralobular reticular fibres) (arrows). Some reticular fibres are coiled (arrowhead). Gomori’s ammoniacal silver staining (bar = 32 μm). (S) Sinusoid blood vessel.

**Figure 2** | Lungs sections of a naturally infected with Leishmania (L) infantum: (A) Lower magnification showing some areas with alpha smooth muscle actin (α-SMA) positive staining in brown evident the parenchyma (Bar = 32 μm). (B) High magnification showing alfa-actin (α-SMA) positive cells (pneumocytes and subepithelial layer) (Bar = 16 μm). (C-D) Note the expression of vimentin in low (C) and high magnification (D). It was always diffuse and localized mainly in the lung parenchyma cells, but always discrete in pneumocytes, unlike of α-SMA. (Bars = 32 and 16 μm, respectively). Immuno-streptavidin-peroxidase method.

**Figure 3** | Kidney tissue sections showing in (A) sclerotic glomerular sclerosis (B) note a diffuse chronic tubulointerstitial fibrosis
The study of interaction between Leishmania and its host via evaluation of IL-8 and IL-23 genes expression

Ali akbar Shamsiyana *, Zahra Navi 1, Abdulrahim Rezaei2, Narges Khazaei 1

1 Department of Parasitology and Mycology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; 2 Department of Immunology, Faculty of Medicine, Mashhad University of Medical Sciences, Iran.

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Abstract

Leishmaniasis is a protozoan infection which is mostly self-healing. Yet, in some cases lesions may spread because of lack of host resistant. The important immunologic indicators, involving the healing of CL in humans are not completely known. Cell-mediated immunity (CMI) plays an important role. The accessory cells and immune cells activity determine the healing or deterioration outcomes. For example, an appropriate increase in Th1 response against Leishmania infection is protective, while an increase in Th2 may worsen the signs and symptoms of disease [1]. In this study IL-8 and IL-23 genes expression in infected people by Leishmania major were compared with no infected people. Samples were prepared from 9 healthy (Leishmaniasis, HIV, HTLV1, HBV negative) individuals and 23 CL patients. 10 cc vein blood was taken, Peripheral blood mononuclear cells (PBMCs) were isolated and RNA was extracted and reverse transcribed to cDNA, Then Real time PCR was conducted and level of IL-8 and IL-23 expression were measured by, Taq Man method before and after PHA stimulation of PBMCs. The results were statistically analyzed using SPSS v 16. The findings of this study indicated that the mean IL-8 gene expression in patients with major leishmaniasis is significantly increased compared with healthy samples, and this increase is significant (Mann-Whitney test, P = 0.038). Also, the mean gene expression of IL-23 in patients with major Leishmaniasis is higher than healthy subjects, but this increase is not significant. Comparison of the IL-8 gene expression level with the location of the lesion by Wilcoxon test showed that the IL-8 gene expression level was correlated with the location of the lesions. Particularly, patients who had face and hand ulcers indicated the most increase of IL8 level. At the end we can conclude that IL8 secretion which is correlated with neutrophil recruitment could be account for the first defense line of acquired and innate immunity in leishmaniasis. In addition, face and hand lesions are more stimulating and provoke immune system more effective. In comparison with IL8, IL23 is less important and shows slight difference among uninfected and infected individuals.

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Correspondence: Email - shamsiyana@mums.ac.ir
Impact of miltefosine resistance and miltefosine treatment on virulence of *Leishmania infantum*

Eline Eberhardt¹, Dimitri Bulté¹, Lieselotte Van Bockstal¹, Sarah Hendrickx¹, Louis Maes³, Guy Caljon¹

¹Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Antwerp, Belgium.

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**Abstract**

Miltefosine (MIL) is the only oral drug for visceral leishmaniasis and although deficiency in an aminophospholipid/miltefosine transporter is sufficient to elicit drug resistance, only very few naturally MIL-resistant (MIL-R) parasites have yet been isolated. To study the impact of MIL resistance acquisition, two isogenic bioluminescent lines of *Leishmania infantum* that originated from a French HIV patient (MHOM/FR/96/LEM3323) were selected for differential susceptibility to MIL (MIL-S and MIL-R) [1], followed by transfection to express red-shifted luciferase (PpyRE9) which is a proficient tool for downstream in vivo bioluminescent imaging.

Experimental induction of MIL resistance, which was linked to a frameshift mutation in the MIL transporter [2], resulted in a reduced *in vitro* promastigote growth rate and a reduced metacyclogenesis. MIL-R parasites were still capable of infecting macrophages *in vitro* but displayed a decreased intracellular multiplication and a severely compromised virulence in mice. The MIL-S parental line produced maximal bioluminescent signals in liver, spleen and bone-marrow corresponding to a typical visceral infection progression, whereas MIL-R only showed a limited bioluminescent signal in the liver that disappeared by 3-4 wpi. This compromised dissemination of the MIL-R strain was associated to a high induction of pro-inflammatory cytokines such as IFN-γ, IL-6 and TNF-α during the early onset of infection. Co-infection studies illustrated that MIL-S can outcompete MIL-R *in vivo* and episcopal reconstitution with the wildtype MIL transporter was able to restore parasite virulence. Surprisingly, *in vivo* MIL-treatment or *in vitro* MIL-pre-exposure significantly rescued MIL-R parasite virulence in all target organs. *In vitro* exposure of MIL-R promastigote cultures to MIL modified the parasite morphology into a longer more slender shape, suggesting an altered parasite membrane composition.

Collectively, this experimental study demonstrates a detrimental impact of a non-functional MIL transporter on virulence/ infectivity in mice. MIL-treatment of these parasites partially reverses this fitness loss and enables *in vivo* growth. These observations emphasize the importance of MIL-resistance profiling prior to drug administration.

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**References:**


**Correspondence:** Email - guy.caljon@uantwerpen.be
In vitro drug susceptibility assessment of clinical isolates: which cell type to use?

Sarah Hendrickx 1, Guy Caljon 1, Louis Maes 1

1 Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Antwerp, Belgium.

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Abstract

Monitoring drug susceptibility of clinical Leishmania isolates still relies on standard in vitro drug-susceptibility assays. Although such assay is extensively used in laboratories all over the world, no standardized protocol is yet available resulting in the use of different parasite stages, a wide variety of host cells (both primary cells and cell lines), associated manipulations, diverse drug exposure times, detection methods and endpoint criteria. In the past, pleas have already been made for standardization to decrease inter-laboratory variation and to enable a more direct comparison of results [1]. Starting from the in vitro standard susceptibility assay on intracellular amastigotes, agreement should be reached at least on which host cell to use.

The advantages and disadvantages of using a number of primary cell types and cell lines were compared by screening their initial receptivity to four strains of Leishmania infantum promastigotes and their role to support intracellular multiplication. Additionally, the impact of primary cell stimulation and cell adherence time was explored for mouse primary peritoneal macrophages. An overall assessment of the cell's accessibility, manipulability and manageableability was made, aiming to advocate one particular cell type that supports the most cheap, easy, rapid and straightforward drug susceptibility procedure.

Despite their proven differences in infection receptivity and support in vivo, primary cells isolated from Swiss, BALB/c and C57BL/6 mice only showed minor alterations in terms of receptivity to infection, dependent on different cell stimulation procedures and cell adherence times. In addition, a few dissimilarities were noted between the different cell types with regard to support of intracellular multiplication.

The more practicable (e.g. cheapest) and suitable in vitro assay to measure drug susceptibility of clinical isolates involves primary peritoneal macrophages derived from Swiss mice obtained after 24h stimulation with starch and allowed to establish in culture for 48 hours before infection. Incubation takes 120h without change of the culture medium and overall parasite burdens are assessed microscopically upon Giemsa staining. In situations where primary cells are not accessible, THP1 cells represent the most pragmatic choice.

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Correspondence: Email - louis.maes@uantwerpen.be
New World cutaneous leishmaniasis in a traveller to a country endemic for Old World cutaneous leishmaniasis

C M Vassalos, G Spanakos, A Vakali, A Mpimpa, N Tegos, E Vassalou

1 Greek Health System/Technological Institute of Athens; 2 Hellenic Centre for Disease Control and Prevention; 3 Department of Parasitology, Entomology and Tropical Diseases, National School of Public Health

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ABSTRACT

Cutaneous leishmaniasis (CL) is caused by both Old World (OW) and New World (NW) Leishmania species. We present an uncommon case of NWCL in a US tourist to Greece that is endemic for OWCL [1]. A 23-year-old female traveller to Greece was referred for laboratory evaluation of a non-healing, painless, 1.3-cm crusted ulcerative lesion on the left leg. She had presented to a local physician and received topical antibiotics. She recalled the first appearance of the lesion three weeks ago, while touring the Greek countryside. We removed the crust and scraped material from the lesion edge. Smear microscopic examination disclosed the presence of Leishmania, confirmed by a Leishmania genus-specific PCR. The PCR product was not cleaved by Apol, thus excluding L. infantum as the causative agent, and indicating the possible presence of L. tropica, which is the most common cause of CL in Greece [2]. We asked our patient about her itinerary. She has been in the country only for two months in a period not corresponding to the seasonal activity period of sand flies in Greece, but she had been to Costa Rica two months before arriving in Greece. PCR product was subsequently sequenced. The sequence was identical to that of L. panamensis strain MHOM/PA/94/PSC-1. Therapy with miltefosine was proposed, as NWCL may progress to mucocutaneous disease in contrast to often self-limited OWCL. Travellers with CL coming to a setting endemic for CL may be infected with Leishmania species that are common in their previous travel destinations. In these cases, molecular methods need to be employed as speciation is the key to choose an adequate treatment option as therapeutic recommendations differ according to species.

Figure 1 (a) Crusted ulcer on traveller’s left leg. b). Amastigotes in macrophage. c) Lane 1: gel electrophoresis of the PCR product after digestion with the restriction endonuclease Apol. M: Size marker (100 bp DNA ladder).

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Correspondence: Email - vassalos.constantine@gmail.com

Maykon Tavares de Oliveira 1*, Carlos Alessandro Fuzo 1, Sandra Regina Costa Maruyama 1, Allynne Karen Mendonça de Santana 1, José Marcos Ribeiro 1, Vanessa Carregaro 1, Amélia Ribeiro de Jesus 1, Roque Pacheco de Almeida 1, João Santana da Silva 1.

1 Programa de Pós-graduação em Imunologia Básica e aplicada, Laboratório de Imunoparasitologia, Faculdade de Medicina de Ribeirão Preto – FMRP/USP, Universidade de São Paulo

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Abstract

Leishmaniasis is a cluster of diseases caused by protozoa of the genus Leishmania and affects more than 12 million people worldwide, with 310 million people at risk of infection. In Brazil, L. infantum is the etiological agent of visceral leishmaniasis (VL), which presents a chronic, life-threatening, and widespread pathology. Among the infected individuals, 85% remain asymptomatic, while the remaining 15% present clinical manifestations, ranging from oligosymptomatic (mild) to more severe symptomatic forms, leading to death. We believe that the quality of the immune response generated by the host determines the final clinical outcome of the disease and that it would be directed by specific transcriptional profiles. Thus, our aim was characterize the peripheral blood transcriptomes of symptomatic infected individuals before and after conventional treatment, as well as of asymptomatic individuals, in order to obtain an overview of the genes involved in the disease pathogenesis. For this, the transcriptional profile was obtained through RNA-seq on the Illumina platform and analyzed by bioinformatics tools, which identified molecular processes involved in the immune response generation and / or modulation of different clinical outcomes of VL caused by L. infantum. This analysis enabled the discovery of ten pathways and several important gene targets, which may in future serve as therapeutic targets and biomarkers for the treatment and prognosis of Visceral Leishmaniasis.

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Correspondence: Email - maykontavares@yahoo.com.br