ABSTRACTS

Monday, December 17, 2012

Workshop: Chemotherapy and Prevention of Parasitic Diseases

08:00 – 09:00 **Registration**

09:00 -10:45 **Session 1: Malaria and Giardiasis**

Chairs: Hagai Ginsburg & Esther Marva

09:00 – 09:25 **Possible mechanism of hypotension in severe malaria -** Nicholas H. Hunt, Yutang Wang, Hanzhong Liu, Gavin McKenzie, Paul K. Witting, Ben J. Wu, Helen J Ball, Shane R. Thomas, John F. Keaney, Jr & Roland O. Stocker

09:25 – 09:45 **Immunomodulator and anti-plasmodial drug combinations for treatment of cerebral malaria (CM) -** Jacob Golenser, Judith Waknine-Grinberg, Haim Ovadia, Gabriele Schramm, Helmut Haas, Jintao Guo, Nicholas H. Hunt, Yechezkel Barenholz

09:45 – 10:00 **Artemether-Lumefantrine (Coartem®) compared with Atovaquone-proguanil (Malarone®) as a treatment for Plasmodium falciparum Malaria in travelers -** Shirly Grynberg & Eli Schwartz

10:00 – 10:15 **The contemporary epidemiology of giardiasis in Israel -** Eran Kopel, Chantal Sadik, Esther Marva & Emilia Anis

10:15 – 10:30 **Giardiasis treatment failure – insight from case studies in travelers -** Eyal Meltzer & Eli Schwartz

10:30 – 10:45 **Impaired parasite attachment as fitness cost of metronidazole resistance in *Giardia lamblia* -** Noa Tejman-Yarden, Maya Millman, Tineke Lauwaet, Barbara J. Davids, Frances D. Gillin, Linda Dunn, Jacqueline A. Upcroft, Yukiko Miyamoto & Lars Eckmann

10: 45 - 11:00 ***Giardia duodenalis* in domestic animals: prevalence and zoonotic significance -** Alex Markovics, A., Argentaro, S., Baneth, G. & Kuzi, S.

Possible mechanism of hypotension in severe malaria

Nicholas H. Hunt[1], Yutang Wang[1], Hanzhong Liu[2], Gavin McKenzie[3], Paul K. Witting[1], Ben J. Wu[1], Helen J Ball[1], Shane R. Thomas[2], John F. Keaney, Jr[4] & Roland O. Stocker[1]

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Background: Hypotension, of varying degrees of severity, has been reported in malaria infection of adults or children in Africa, Indonesia and SE Asia. The mechanisms underlying this clinical complication have not been identified.

Methods: To address this issue we used *Plasmodium berghei* ANKA (PbA) infection in mice as a model system. Further investigations were carried out using isolated vessels or endothelial cells from various species, including humans.

Results: Five days post-inoculation (p.i.), PbA-infected mice were hypotensive, confirming previous findings of others. In these mice, indoleamine dioxygenase-1 (IDO-1) was strongly expressed in endothelial cells in many vessels, including resistance vessels. This expression was reflected in increased activity of the enzyme, since plasma concentrations of the substrate, tryptophan, were decreased and those of the product, kynurenine, were increased in PbA-infected mice. A selective inhibitor of IDO-1, 1-methyl-tryptophan, restored systolic blood pressure in PbA infection, but did not influence systolic blood pressure in uninfected mice. IDO-1 expression and activity were induced in cultured endothelial cells taken from arteries and veins of various species. Kynurenine concentrations in the medium reached millimolar concentrations. Tryptophan added to pre-constricted arterial rings caused their relaxation, an effect that was dependent on the presence of endothelial cells and IDO-1 activity. Kynurenine relaxed pre-constricted vessels in the same system, though this effect was endothelium-independent. Kynurenine also decreased blood pressure in spontaneously hypertensive rats.

Conclusion: In severe malaria, interferon-γ, produced by $CD8^+$ T cells in response to the presence of intraerythrocytic malaria parasites, induces the endothelial expression of IDO-1. This converts tryptophan to kynurenine, which, via cyclic GMP-dependent signalling, causes the relaxation of vascular smooth muscle cells in resistance vessels, resulting in hypotension. Parallel studies suggest that a similar mechanism operates in experimental sepsis.

Immunomodulator and anti-plasmodial drug combinations for treatment of cerebral malaria (CM)

Jacob Golenser[1,2], Judith Waknine-Grinberg[1,3], Haim Ovadia[4], Gabriele Schramm[5], Helmut Haas[5], Jintao Guo[6], Nicholas H. Hunt[6], Yechezkel Barenholz[3]

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Cerebral malaria (CM) is the most severe complication of *Plasmodium falciparum* infection, and a leading cause of death in children under the age of five in malaria-endemic areas. CM is likely the result of a complex sequence of interrelated events: mechanical obstruction and upregulation of numerous immune-related responses (especially Th1-type responses), all of which lead to blood-brain-barrier (BBB) breakdown, and damage or death of microglia, astrocytes, and neurons. Using the *Plasmodium berghei* ANKA mouse model for experimental cerebral malaria (ECM) we found that several immunomodulators may decrease or eliminate ECM, including curcumin, fasudil, schistosomal antigens and a novel formulation of liposome-encapsulated glucocorticoids, β-methasone hemisuccinate (BMS).

Encapsulation of BMS in nano-sterically stabilized liposomes (nSSL) dramatically improved BMS efficacy and abolished the high toxicity seen upon administration of free compound. Administration of nSSL-BMS reduced ECM rates in a dose-dependent manner and created a survival time-window needed for administration of an anti-plasmodial drug (before severe anemia is developed). The above treatment lead to lower levels of cerebral inflammation, expressed in corresponding plasma cytokines (protein measurments) and brain chemokines and cytokines (RNA and proteins), reduced hemorrhage and edema. Administration of the liposomal formulation resulted in selective accumulation of BMS in the brains of sick mice. nSSL-BMS effectively prevented the cerebral syndrome even if treatment was started at late stages of the disease, when disruption of the blood-brain barrier had already occurred and clear signs of neurological impairment were present. Administration of the anti-plasmodial artemisone, following nSSL-BMS treatment, resulted in prevention of ECM and complete cure of the malaria symptoms.

Overall, combined immunomodulator/anti-plasmodial treatment may be considered for parasite elimination, and prevention of human CM and long-term cognitive damage.

We thank Prof. Richard Haynes from The Hong Kong University of Science and Technology, for the donation of artemisone.

Artemether-Lumefantrine (Coartem®) compared with Atovaquone-proguanil (Malarone®) as a treatment for *Plasmodium falciparum* malaria in travelers

Shirly Grynberg & Eli Schwartz

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Background: Malaria is a major cause of morbidity and mortality in travelers. Although Israel has been declared malaria free since 1966, there is an increasing number of malaria cases imported to Israel by travelers, immigrants, and foreign workers. *Plasmodium falciparum* is the major cause of severe malaria and its treatment is complicated by the emergence of resistant strains. In the last decade two new drugs have become available for treating *P. falciparum* malaria in Western countries, atovaquone - proguanil (AP, Malarone) and artemether - lumefantrine (AL, CoArtem). Both drugs are considered to be safe and effective and indicated for uncomplicated falciparum malaria, but to date, no study has been conducted comparing the effectiveness of these two drugs. In this study, we compared the effectiveness of these drugs in non-immune Israeli travelers returning with falciparum malaria.

Methods: We performed a retrospective analysis comparing the outcome of patients treated with AP vs. AL. The study was conducted in the Center of Geographic Medicine and Tropical Diseases, at Sheba medical center. Included were patients with P. falciparum who were treated by either AL or AP as a single drug, during the years 2001 to 2012. Patients with severe malaria that received IV medication were excluded, also immigrants from malaria endemic countries and patients who did not receive their treatment in Israel were excluded. The major end-points were treatment failure (recrudescence) and fever clearance time. Data on each patient, including demographic data, fever clearance time, treatment outcome and hospitalization time were extracted from their medical records

Results: In total, 62 patients were included, 45 in the AP group and 17 in the AL group. 58 of the patients acquired the disease in Africa (93% in AP vs. 94% in AL group). Almost all were males (93%). The mean age of the patients was 43 years (41in the AP and 47 in the AL).

Outcome: Treatment failure was observed in 6 of the 45 (13.3%) of the AP group, while there were no treatment failures in the AL group. Furthermore, the average fever clearance time (information was available for 77% of the patients) was significantly lower in the AL group compared to the AP group, 48±20.4 vs. 80 ± 26.6 hours (P=0.01).

Conclusion: Our results showed that AL is more effective than AP in treating *P. falciparum* in a traveler population, and probably should be the drug of choice for treating *P. falciparum* infection also in traveler populations. The high failure rate of AP treatment is alarming.

The contemporary epidemiology of giardiasis in Israel

Eran Kopel[1], Chantal Sadik[1], Esther Marva[2] & Emilia Anis[1]

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Introduction: Giardiasis is a common parasitic diarrheal disease caused by the parasite *Giardia lamblia* that is often waterborne. The infection is by fecal-oral route from person to person or animal to person. Previous studies in Israel, mostly from before 2000, described high prevalence among children of Arab and Bedouin origin, children living in rural areas, and Ethiopian immigrants.
Methods: Giardiasis is a notifiable disease in Israel since 2001. Individual case-notifications are reported by health care providers and clinical laboratories to the District Health Offices. The reports are processed nationally by the Division of Epidemiology and provide a basis for all incidence data and epidemiological analysis of illnesses among civilians. We summarized the nationwide epidemiology of giardiasis in Israel for 2002-2011.
Results: The average annual incidence was 24.2 cases per 100,000 population (range, 19.9 in 2011 to 28.3 in 2007). The average annual incidence by gender was higher in males (27.8 cases per 100,000 males) than in females (20.5 cases per 100,000 females). The average annual incidence by age group was highest in children aged 1-4 (142.7 cases per 100,000) followed by 58.1 cases per 100,000 infants <1 year. The average annual incidence by region was highest in Haifa and Beer Sheva Sub-Districts (both ≈49 cases per 100,000) followed by Tel Aviv District (32.7 cases per 100,000). The average annual incidence by population group was 25.0 cases in Jews and 19.3 cases in Non-Jews per 100,000. The highest incidence was observed during the warm season (July-November; range, 2.3-2.7 cases per 100,000 population per month).
Conclusions: Giardiasis continued to be endemic in Israel in the last decade and substantial morbidity was seen, unexpectedly, in urban settings and among Jews. These findings call for continued surveillance and implementation of appropriate prevention measures.

Giardiasis treatment failure – insight from case studies in travelers

Eyal Meltzer, Eli Schwartz

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Giardiasis is a frequent cause of diarrhea in the developing world. Although the disease is often self-limited, it can cause protracted and sometimes severe diarrhea. It is also the most frequent diagnosed protozoan cause of diarrhea in travelers returning from developing countries. The main chemotherapeutic agents used to treat Giardia are the nitroimidazoles (metronidazole, tinidazole, ornidazole and others), and albendazole. Quinacrine, an effective old drug, is no longer available. Nitazoxanide is a newer agent, has proven efficacy in treating Giardiasis. Studies regarding giardiasis treatment failure in developing countries are problematic because of the possibility of reinfection. Returning travelers, with their low likelihood of reinfection are therefore better subjects for studies on treatment failure in giardiasis. However, until recently, the differentiation between Giardia treatment failure and post-infectious irritable bowel syndrome (PIBS) has been hampered by the low sensitivity of stool microscopy. The advent of highly sensitive and specific stool antigen tests has enabled clinicians a tool in follow-up, and establishing the diagnosis of treatment failure when symptoms persist after treatment. Case studies of such travelers will be presented, with a review of relevant literature and the recommended approach to treatment.

Impaired parasite attachment as fitness cost of metronidazole resistance in *Giardia lamblia*

Noa Tejman-Yarden,[1] Maya Millman,[1] Tineke Lauwaet,[2] Barbara J. Davids,[2] Frances D. Gillin,[2] Linda Dunn,[3] Jacqueline A. Upcroft,[3] Yukiko Miyamoto,[1] and Lars Eckmann[1]*

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Infections with the diarrheagenic protozoan pathogen *Giardia lamblia* are most commonly treated with metronidazole (Mz). Treatment failures with Mz occur in 10 to 20% of cases and Mz resistance develops in the laboratory, yet clinically, Mz-resistant (Mzr) *G. lamblia* has rarely been isolated from patients. To understand why clinical Mzr isolates are rare, we questioned whether Mz resistance entails fitness costs to the parasite. Our studies employed several newly generated and established isogenic Mzr cell lines with stable, high-level resistance to Mz and significant cross-resistance to tinidazole, nitazoxanide, and furazolidone. Oral infection of suckling mice revealed that three of five Mzr cell lines could not establish infection, while two Mzr cell lines infected pups, albeit with reduced efficiencies. Failure to colonize resulted from a diminished capacity of the parasite to attach to the intestinal mucosa *in vivo* and to epithelial cells and plastic surfaces *in vitro*. The attachment defect was related to impaired glucose metabolism, since the noninfectious Mzr lines consumed less glucose, and glucose promoted ATP-independent parasite attachment in the parental lines. Thus, resistance of *Giardia* to Mz is accompanied by a glucose metabolism-related attachment defect that can interfere with colonization of the host. Because glucose-metabolizing pathways are important for activation of the prodrug Mz, it follows that a fitness trade-off exists between diminished Mz activation and reduced infectivity, which may explain the observed paucity of clinical Mzr isolates of *Giardia*. However, the data also caution that some forms of Mz resistance do not markedly interfere with *in vivo* infectivity.

Giardia duodenalis in domestic animals: prevalence and zoonotic significance

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Giardia duodenalis is an enteric protozoan pathogen of domestic animals and humans caused by at least seven distinct genotypes. This parasite may cause a broad range of clinical signs from no signs in asymptomatic animals to acute severe diarrhea. Although *Giardia* infection is commonly diagnosed in the laboratory of Kimron Veterinary Institute in livestock, wild and pet animals, no studies on prevalence and epidemiology of the infection were undertaken until recently. In a study conducted in the last year, the prevalence of *Giardia* infection and possible risk factors were investigated by testing 163 stool samples from dogs admitted to the Koret Veterinary Hospital during a 3 year period. The samples were tested for *Giardia* antigen by a commercial ELISA test (FASTest® GIARDIA, Mega Cor, Austria). The overall prevalence was 11.9%. The prevalence rate was significantly higher in young dogs less than one year old (20.8%) compared to older dogs (7.5%).The prevalence of *Giardia* infection was also higher among the kennel dogs (16,7%). Examination of fecal samples by zinc sulfate flotation for *Giardia* cysts of 5009 dogs and 233 cats submitted to the Kimron Veterinary Institute for routine parasitological diagnosis during the last five years revealed infection rates of 1.5% and 3%, respectively. *Giardia* cysts were detected in higher rates from dogs in most kennels and dog shelters, from zoo animals and from dairy calves. These results, the prevalence and the zoonotic significance of animal giardiasis are discussed.

11:30- 13:00 **Session 2: Leishmaniasis**

Chair: Charles Jaffe & Lionel Schnur

11:30 – 11:50 **Liposomal Amphotericin B in comparison to Sodium Stibogluconate for *Leishmania braziliensis* cutaneous leishmaniasis in travelers** - Schwartz E, Solomon M, Pavlotzki F & Barzilai A.

11:50 – 12:05 **Treatment of *Leishmania tropica* in children: A retrospective study -** Michal Solomon, Eli Schwartz, Felix Pavlotsky, Aviv Barzila & Shoshana Greenberger

12:05 – 12:25 **Hypoxia impairs the NO-dependent leishmanicidal activity of macrophages and prevails in the skin lesions of *Leishmania major*-infected mice -** Alexander Mahnke, Robert M. Meier, Valentin Schatz, Kirstin Castiglione, Andrea Debus, Heidi Sebald, Ulrike Schleiche, Otto Wolfbeis, Christian Bogdan & Jonathan Jantsch

12:25 – 12:45 **Development of Drugs Against Leishmaniasis Using a Rational Approach** - Nir Qvit, Danilo Ciccone Miguel, Vivian I. Bonano, Deborah Schechtman, Silvia R. B. Uliana & Daria Mochly-Rosen

12:45 – 13:00 **Cutaneous leishmaniasis in Sde Eliyahu: Deciphering the transmission cycle** - Faiman, R., Abbasi, I., Jaffe, C.L., Motro, Y., Nasereddin, A., Schnur, L.F., Torem, M. Pratlong, F., Dedet, J-P. & Warburg, A.

13:00 - 14:00 **Lunch**

Liposomal Amphotericin B in comparison to Sodium Stibogluconate for *Leishmania braziliensis* cutaneous leishmaniasis in travelers

Schwartz E, Solomon M, Pavlotzki F & Barzilai A.

The Center of Geographic Medicine and Department of Dermatology, Sheba Medical Center, Tel Hashomer

Background: New World Cutaneous Leishmaniasis is mostly acquired in the Amazon Basin of Bolivia where *L. viannia (V.) braziliensis* is endemic. Treatment with systemic pentavalent antimonial compounds is effective in achieving clinical cure in only 75% of the cases. We assessed the efficacy and safety of Liposomal Amphotericin B (L-Amb) treatment for primary infection of cutaneous *L. (V.) braziliensis.*

Methods: A prospective observational evaluation was performed for Cutaneous Leishmaniasis due to *L. (V.) braziliensis,* which was treated with L-Amb 3 mg/kg for 5 consecutive days, and a 6^{th} dose on day 10. This therapy regimen was compared to the treatment regimen of Sodium Stibogluconate (SSG) 20 mg/kg for 3 weeks.

Results: Our study was divided into two groups; 34 patients received L-Amb and 34 received SSG treatment. Almost all patients were infected in Bolivia. In the L-AmB group, 29 (85%) patients had complete cure compared to 70% in the SSG group (P=NS), 4 other patients were slow healers and only 1 patient needed additional treatment with SSG. No relapses were seen during a mean 29 month follow up period. Failure rate was 3% in the L-Amb vs. 29% in the SSG group (p=0.006). Treatment was interrupted in 65% of patients on SSG due to adverse events while all patients receiving L-Amb, completed the treatment.

Conclusions: L-Amb treatment for *L. (V.) braziliensis* is effective, better tolerated and more cost beneficial. L-Amb should therefore be considered as the first-line treatment option for cutaneous *L. (V.) braziliensis* infection.

Treatment of *Leishmania tropica* in children: A retrospective study

Michal Solomon MD[1], Eli Schwartz MD, DTMH[2], Felix Pavlotsky MD[1], Aviv Barzilai MD, MSc[1], Shoshana Greenberger MD,PhD[1]

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Background: Cutaneous leishmaniasis (CL) is endemic in Israel and has been attributed almost exclusively to *Leishmania major*. Over the last decade, there have been increasing reports of CL due to *Leishmania tropica* in several regions of Israel. Children represent a large portion of the affected patients and a challenge to the treating clinician because of low compliance to topical injections and limited data on safety and efficacy of systemic therapy.
Objective: To characterize the clinical presentation and evaluate the efficacy and safety of topical and systemic treatments in pediatric patients affected with *L. tropica*.
Patients and methods: A retrospective single center study was performed on 47 children with *L. tropica* CL in an outpatient setting. Treatment included topical therapy (Paromomycin ointment, Intra-Lesional (IL) injection of Pentavalent Antimony, Cryotherapy) or systemic therapy (Liposomal Amphotericin B(L-Amb) or Pentavalent Antimony)
Results: Between the years 2008 to 2012, 70 patients with L. tropica were treated at our center, 47 (67%) of them were children. Average age was 8.8 years (range 1-15). The face was the most common site of involvement (79%). Average number of lesions was 2.6 (range 1-10). Twenty-four children (51%) required systemic therapy due to failure of topical treatment, involvement of the face or multiple lesions. Patients were treated with IV L-Amb, 3-5 mg/kg. Of these, full response was observed in 83% within 3 months. Serious adverse effects were not reported. Four children did not respond to L-amb and were subsequently treated with systemic Pentavalent Antimony with good response.
Conclusion: The disease burden of L. Tropica in children is high. Due to facial involvement and low response to topical therapies, systemic therapy is often required. Systemic therapy with L-Amb is safe, effective and much shorter compared to Pentavalent Antimony. In cases of L-Amb treatment failure Pentavalent Antimony can be an effective second line therapy.

Hypoxia impairs the NO-dependent leishmanicidal activity of macrophages and prevails in the skin lesions of *Leishmania major*-infected mice

Alexander Mahnke[1], Robert M. Meier[2], Valentin Schatz[1], Kirstin Castiglione[1], Andrea Debus[1], Heidi Sebald[1], Ulrike Schleicher[1], Otto Wolfbeis[2], Christian Bogdan[1], Jonathan Jantsch[1]

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Introduction: Cure of infections with *Leishmania* (*L.) major* is critically dependent on the activity of the inducible NO synthase (NOS2) that produces high levels of NO from L-arginine in the presence of ample oxygen. Therefore, we hypothesized that the tissue-oxygenation in cutaneous lesions may be an important factor that affects the leishmanicidal activity of macrophages.
Methods: C57BL/6 mice were infected with *L. major* promastigotes in the footpad. Transcutaneous oxygen imaging as well as pimonidazol immunohistochemistry was used to determine the pO_2 in *L. major*-infected skin lesions. In order to assess the influence of oxygen on the parasite burden *in vivo*, we placed mice in a hypoxia chamber (pO_2 approx. 9.0 %). Bone marrow-derived macrophages from *Nos2*$^{-/-}$ and wild-type mice infected with *L. major* parasites were analyzed for their leishmanicidal activity under different atmospheric pO_2. NO release was estimated by the measurement of nitrite accumulation in the culture supernatants using the Griess-Assay.
Results: When *L. major* skin lesions reached their maximum size, the infected tissue was clearly hypoxic (pO_2 approx. 2.8%). At an atmospheric pO_2 below 4%, macrophages activated *in vitro* by IFN-gamma plus LPS were unable to produce NO and to clear intracellular *L. major*. However, upon reoxygenation to normoxic conditions these activated macrophages produced NO again and efficiently cleared the parasites. Infection of activated *Nos2*$^{-/-}$ macrophages under normoxic conditions completely mimicked the defective leishmanicidal activity observed in infected wild-type macrophages under hypoxic conditions. Using pharmacological NO-donors we were able to rescue the defective leishmanicidal activity observed in hypoxic *L. major*-infected macrophages. Furthermore, our data demonstrate that systemic hypoxia impairs the ability of infected animals in a *Nos2*-dependent manner to clear *L. major*.
Conclusions: This study represents the first quantitative assessment of the pO_2 in *L. major* skin lesions and demonstrates that the lesions are hypoxic. Since atmospheric hypoxia impaired the leishmanicidal activity of activated macrophages in an NO-dependent manner *in vitro* and the ability of infected mice to clear *L. major in vivo*, we propose that hypoxia may be a hitherto underestimated local milieu factor which contributes to the persistence of *Leishmania* even in immunocompentent hosts.

Development of drugs against leishmaniasis using a rational approach

Nir Qvit[1], Danilo Ciccone Miguel[2], Vivian I. Bonano[2], Deborah Schechtman[3], Silvia R. B. Uliana[2], Daria Mochly-Rosen[1]

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For over 20 years, we have developed short peptide inhibitors of protein-protein interactions between signaling enzymes, such as protein kinase C (PKC), and its scaffold protein, receptor for activated C-kinase (RACK). These short bioactive peptides are highly selective and effective in several animal models of human diseases. Some of these peptides were tested in humans and were shown to be safe. RACK interacts with and regulates multiple signaling enzymes that have key cellular functions. The RACK ortholog in *Leishmania*, called LACK, is not well characterized but is functionally critical. Parasites in which LACK was knocked out are not viable, and parasites that express low levels of LACK fail to parasitize even immune-compromised mice. Because of its homology to RACK, we assumed that LACK also interacts with multiple signaling enzymes in the parasite and might be a key scaffolding protein involved in essential signaling processes. Furthermore, LACK is found in both amastigotes and promastigotes of *Leishmania*. Therefore, we predicted that LACK is a good drug target, and we developed novel peptides aimed at inhibiting LACK interactions with LACK-binding proteins. Peptides were developed based on a sequence homology search and structural studies and were conjugated to TAT-derived peptide for drug delivery. When used to treat *L. amazonensis* promastigote cultures for 24 hours, some peptides resulted in growth inhibition with IC_{50} of approximately 10 μM. Furthermore, these peptides inhibited infection of macrophages by *L. amazonensis* promastigotes. The peptides are non-toxic to macrophages. Therefore, without any knowledge on partner proteins of LACK, we were able to design presumed inhibitors of LACK's function and affect the parasite's viability. Our method is likely applicable to design other anti-parasitic drugs.

Cutaneous leishmaniasis in Sde Eliyahu: Deciphering the transmission cycle

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In 2006/7 18 cases of cutaneous leishmaniasis (CL) were reported for the first time from Sde Eliyahu (pop. 650), a village in the Beit She'an valley of Israel. Between 2007-2011 a further 88 CL cases were diagnosed bringing the total to 106 (16.3% of the population). The majority of cases resided in the south-western part of the village along the perimeter fence. The causative parasite was identified as *Leishmania major* Yakimoff & Schokhor, 1914 (Kinetoplastida: Trypanosomatidae). *Phlebotomus papatasi* (Scopoli), 1786 (Diptera: Psychodidae) was found to be the most abundant phlebotomine species comprising 97% of the sand flies trapped inside the village, and an average of 7.9% of the females were positive for *Leishmania* ITS1 DNA. Parasite isolates from CL cases and sand flies were characterized using several methods and shown to be *L. major*. During a comprehensive survey of rodents 164 Levant voles *Microtus guentheri* Danford & Alston, 1880 (Rodentia: Cricetidae) were captured in alfalfa fields bordering the village. Of these 27 (16.5%) tested positive for *Leishmania* ITS1 DNA and shown to be *L. major* by reverse line blotting. A very high percentage (58.3% - 21/36) of Tristram's jirds *Meriones tristrami* Thomas, 1892 (Rodentia: Muridae), found further away from the village also tested positive for ITS1 by PCR. Isolates of *L. major* were successfully extracted from the ear of a jird found positive by ITS1 PCR. Although none of the wild PCR-positive voles exhibited external pathology, laboratory-reared voles that were infected by intradermal *L. major* inoculation, developed patent lesions and sand flies became infected by feeding on the ears of these laboratory-infected voles. This is the first report of *Leishmania* isolation from *M. tristrami* and the implication of *M. guentheri* as reservoir of *Leishmania*. The widespread co-distribution of *M. guentheri* and *P. papatasi*, suggests a significant threat from the spread of CL caused by *L. major* in the Middle East, central Asia and southern Europe.

14:00 - 15:45	**Session 3: Helminthes and Ectoparasites**
	Chairs: David Hassin & Kosta Y. Mumcuoglu
14:00 – 14:15	**Strongyloidiasis: A case report** – Gut Ledergor
14:15 – 14:30	**Challenges in the diagnosis of strongyloidiasis in Israel -** Tamar Grossman, Guy Ledergor, Noa Kostiner, Moshe Ephros & Esther Marva
14:30 – 14:45	**Strongyloides hyperinfection in Israel -** Eyal Nadir & Oren Zimhony
14:45 – 15:00	**The Treatment of *Diphyllobothrium latum* - More of the same -** Tamar Gottesman, Orit Yossepowitch & Michael Dan
15:00 – 15:15	**The use of insect repellents and related personal protection measures to prevent vector borne diseases -** Laor Orshan
15:15 – 15:35	**New treatment modalities for head lice and scabies –** Kosta Y. Mumcuoglu
15:35 – 15:45	**Discussion**

Strongyloidiasis: A case report

Gut Ledergor

and

Challenges in the diagnosis of strongyloidiasis in Israel

Tamar Grossman [1], Guy Ledergor[2], Noa Kostiner[3], Moshe Ephros[4] & Esther Marva[1]

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Preliminary data from a study examining the causes of eosinophilia in immigrants from Ethiopia indicate that prevalence of Strongyloides infection may be as high as 40%. Laboratory detection includesserology, RT-PCRand microscopy of fresh stool. Missing the diagnosis of strongyloidiasis may endanger patients, especially when treated with immunosuppressive drugs. We present a case of a 36 year-old Israeli man who visited Egypt briefly 4 years prior to the clinical presentation of chronic severe eosinophilia. The patient had no evidence of end-organ damage, and underwent through extensive workup for hypereosinophilic syndrome, until the diagnosis of strongyloidiasis was made. The possibility of infection with Strongyloides in travelers and immigrants with or without eosinophilia should be considered when exposure may have occurred.Diagnosisshould be attempted, and treatment given when infection is confirmed.

Strongyloides hyperinfection in Israel

Eyal Nadir and Oren Zimhony

Infectious Diseases unit, Kaplan Medical Center, Rehovot, Israel.

Strongyloides is an intestinal nematode infecting millions of people worldwide. It can inhabit the GI tract for decades due to its ability to reproduce within the human host (auto-infection). Infection is usually asymptomatic, however, immunosuppressive state of varies causes poses a risk for infected individuals for developing fulminant illness. While hyperinfection refers to accelerated autoinfection, disseminated infection refers to migration of larvae to distant organs. At least seven patients were diagnosed with Strongyloides hyperinfection in our institution during the past decade. All of them were immigrants of Ethiopian origin. All of them had at least one documented risk factor for hyperinfection. Four patients had meningo-encephalitic symptoms upon presentation. In Four patients a GI-tract typical bacterium was isolated from an extraintestinal site. Two patients were pregnant. Only two patients survived. Most patients had well-documented eosinophilia in their past medical forms while none had eosinophilia during the hyperinfection. Chronic Strongyloides can mimic bronchial asthma. Thus, there are many Ethiopian immigrants diagnosed as asthmatic based on clinical presentation only and therefore treated wrongly with corticosteroids. Paradoxically, this mal-treatment makes them more prone to hyperinfection. In order to prevent further fatal cases, it is proposed that every Ethiopian immigrant with asthma and/or chronic eosinophilia should be tested for strongyloidosis. Additionally, all immunosupressed Ethiopian patients should be screened as well, and even receive empirical treatment based only on eosinophilia on their past medical records. The treatment of choice for Strongyloides, either asymptomatic or hyperinfection, is oral Ivermectin, while Albendazole is considered less effective. Nevertheless, whenever a meningo-encephalitic Strongyloidosis is suspected, it should be considered that Albendazole penetrates readily the blood-brain barriers while Ivermectin does not. The use of veterinary formulation of intravenous Ivermectin, subcutaneous injections of Ivermectin and Ivermectin enemas is currently experimental and limited to case reports only, though should be considered in cases of critical illness.

The Treatment of *Diphyllobothrium latum* - More of the same

Tamar Gottesman, Orit Yossepowitch, Michael Dan

Infectious Diseases Unit, E. Wolfson hospital, Holon

Background. The fish tapeworm, *Diphyllobothrium latum*, is exceedingly rare in Israel**,** and the few cases reported were in individuals originating in endemic areas (which include Siberia, Europe - especially Scandinavia and other Baltic countries -, North America, Japan, Chile and Uganda). Human infection with *D. latum* is acquired by eating uncooked freshwater fish containing the parasite's plerocercoid cysts, or consumption of dried or smoked fish, which may contain viable cysts. Infection is usually asymptomatic, but a proportion of infected individuals report nonspecific symptoms such as weakness dizziness, diarrhea, and intermittent abdominal discomfort**.** The diagnosis is often suspected when a patient reports seeing segments or the worm in stool. Prolonged or heavy *D. latum* infection may cause vitamin B_{12} deficiency, which rarely leads to megaloblastic anemia and/or neurologic manifestations. Diagnosis is established by documenting proglottids (reproductive segments of the worm) in stool or by detecting operculated parasite eggs on microscopic stool examination. Treatment with praziquantel **(**Biltricide®, Bayer) is highly effective if administered in adequate dosage.

Case description. 40-year-old women immigrated to Israel from Siberia in 2000. In her country of origin she used to eat uncooked freshwater fish. In 2002 she noticed for the first time segments of worm in her stool. The diagnosis was established in 2007 by identifying *D. latum* eggs in stool (Maccabi Laboratories, Rehovot), and a single oral dose of praziquantel, 600 mg was prescribed. However, the patient continued to excrete eggs and proglottids in her stool. She was referred to our clinic in 2012 for treatment failure. Review of major references (Mandell textbook of infectious diseases, The Sanford Guide® to Antimicrobial Therapy, The Medical Letter, and The DPDx - CDC parasitology diagnostic website) found that the recommended treatment is a single dose of praziquantel, 5-10 mg/kg which is said to have an excellent activity against *D. latum* and most other tapeworm. Accordingly, the patient had apparently received the right drug in the appropriate dosage (6 mg/kg, within the recommended dose range). However, in a recent Update on the Human Broad Tapeworm (Clin Microbiol Rev 2009; 22:146) a higher dose is recommended: 25 mg/kg. Moreover, the authors emphasize that a lower dose of 10 mg/kg showed a poor effect against *D. latum* in experimentally infected golden hamsters. The patient was retreated with praziquantel in April 2012, this time at the suggested higher dose. Since, no more proglottids were seen in stool (last follow-up, October 2012), and repeat stool examinations for parasite eggs were negative.

Discussion. Only one case of human *D. latum* infection was previously reported in Israel (Palestine) in the modern era. It was published in 'Harefuah' in 1934 and is quite similar to ours: A 60-year-old women born in Leningrad, immigrated to Palestine two years prior to her complaints which included the presence of worms in stools without any other symptoms. The diagnosis was established by recovery of *D. latum* eggs in stool. The only additional documentation of *D. latum* eggs in Palestine dates back to the Medieval era (Int'l J Paleopathology 2011;1:132): eggs were found in soil sediment from the excavation of a cesspool in the city of Acre. Archaeological stratigraphy and radiocarbon dating of a fragment of animal bone from the cesspool confirmed its use in the 13th century CE, during the crusader period. It is rare to find tapeworm eggs in archaeological sites in the mainland Near East. The presence of fish tapeworm eggs in a crusader period

cesspool in Acre suggests its use by crusaders or pilgrims from northern Europe who travelled to the Levant carrying these parasites in their intestines.
Although *D. latum* infection remains extremely rare in Israel and is usually imported from endemic areas, it is easily diagnosed and responds well to praziquantel if administered at a dose higher than the one recommended by most therapeutic references.

The use of insect repellents and related personal protection measures to prevent vector borne diseases

Laor Orshan

Laboratory of Entomology, Ministry of Health, Jerusalem

Blood sucking arthropods are major vectors of infectious pathogens like viruses, bacteria, protozoa and nematodes that cause disease in people. Methods and products to protect individuals or small group from nuisance and infecting arthropods bites are an important component of integrated vector control programs and in many circumstances the only available means. The methods and products commonly included in the broad definition of personal protection measures (PPM) are adopting preventive behavior, application of topical repellents directly to the skin, insecticide-treated clothing and bed-nets and various devices that emit insect repelling compounds into a small space. The scientific literature on the effectiveness of PPM is mainly limited to studies of protection from bites. The assumption that the use of effective PPM prevents disease because their use reduces the number of arthropod bites is repeated in the literature and endorsed by the U.S. governmental agencies (EPA and CDC). Few controlled studies have demonstrated some reduction in incidence of vector-borne diseases as an outcome after the use of skin repellents; insecticide treated bed-nets and impregnated clothing. Despite the great potential of PPM to protect people from biting arthropods, their effectiveness in disease prevention requires high level of cooperation, correct and persistence use over time.

New treatment modalities for head lice and scabies

Kosta Y. Mumcuoglu

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Despite the use of powerful insecticides and prodigious efforts of parents and health providers, the number of cases of head louse infestations in Israel, remain high. Successful control can not be achieved due to sale of ineffective pediculicides, the use of alternative, "natural" remedies and methods, whose efficacy has not been proved in in-vitro and in-vivo studies and the development of resistance to insecticides. In Israel, pyrethroids such as permethrin and allethrin as well as malathion, dimethicone and isopropyl myristate based pediculicides are used for control. In addition, there are a large number of alternative ('natural") remedies, which are mainly based on essential oils. Dimethicone based products have the advantage of being physically acting, non-toxic and cosmetically acceptable, some of them should be used only once for 15 min as they are killing all living lice and eggs on the scalp after a single application. They could be used for children over 6 months old, by pregnant and lactating woman and by asthmatic people. Lately, topical and systemic applications of ivermectin have been introduced for the treatment of head louse infestation.

Scabies is a contagious ectoparasitic infestation, which usually spreads by sexual contact, close personal contact with a person who has scabies and close contact with infected clothing, bedding or towels. Permethrin based products are used as the treatment of choice, while lately oral ivermectin has been introduced as an effective and cost-comparable alternative to topical agents in the treatment of scabies infection. It is especially useful in the treatment of severely crusted scabies lesions in immuno-compromised patients, or when topical therapy has failed. Oral dosing may be more convenient in institutional outbreaks and in the treatment of mentally impaired patients. In Israel, ivermectin is permitted in veterinary medicine but not in the treatment of head lice or scabies.

Tuesday, December 18, 2012

08:00 – 09:00	**Registration**
09:00 -10:30	**Plenary lectures**
	Chairs: Eli Schwartz & Jacob Golenser
09:00 – 09.30	**Prospects for prevention and control of malaria -** Nicholas H. Hunt (Australia)
09:30 – 10:00	**Dengue: A hard nut to crack -** Annelies Wilder-Smith (Germany)
10:00 – 10:30	**Giardiasis -** Christoph F.R. Hatz (Switzerland)
10:30 - 10:45	**General assembly**
	Honoring Prof. Eugenie Pipano
10:45 - 11:15	**Coffee break**
11:15 - 13:00	**Parallel sessions (1 and 2)**
13 :00 – 14 :00	**Lunch**
14 :00 – 16:00	**Parallel sessions (3 and 4)**

Prospects for prevention or control of malaria

Nicholas H. Hunt

School of Medical Sciences and Bosch Institute, University of Sydney.

Malaria remains an infectious disease that has enormous adverse effects on human health and on the economic wellbeing of those countries in which it is prevalent. A wide range of organisations and initiatives, including WHO, The Global Fund to Fight AIDS, Tuberculosis and Malaria, The Medicines Against Malaria Venture and The Bill and Melinda Gates Foundation, are working towards the elimination of malaria. Whether achieving this goal is a realistic prospect in the foreseeable future remains to be seen. Important steps on this path will include reducing the incidence of the disease and improving the availability and efficacy of its treatment.

Three important areas of current research can be summarised as follows.

1. Prevention of transmission

   Several different approaches are being taken, including:

   - Molecular engineering of mosquitoes to reduce or eliminate their efficiency as vectors
   - The use of insecticide-impregnated bednets to limit the incidence of nocturnal mosquito bites
   - The development of anti-parasite vaccines.

2. Improved anti-malarial therapy

   The focus here is on:

   - Improved diagnosis of infections
   - The development of new anti-malarial agents
   - Diminishing the importance of drug resistance, e.g. through drug combinations
   - The application of Intermittent Protective Treatment to decrease the incidence of the disease among certain populations or in specific geographical locations.

3. Minimising the morbidity and death resulting from malaria infection

   Strategies include:

   - "Anti-disease" vaccines that decrease morbidity and mortality without necessarily preventing infection
   - Adjunctive therapies, e.g. the use of arginine to counteract endothelial dysfunction
   - Interference with metabolic pathways that lead to malaria complications, e.g. the kynurenine pathway of tryptophan metabolism
   - Development of immunomodulators to reduce the immunopathological processes that cause some of the damaging disease complications.

The focus of this presentation will be on the second and third areas.

Dengue: A hard nut to crack

Annelies Wilder-Smith

University Hospital Heidelberg, Institute of Public Health, Heidelberg, Germany

Dengue virus is the most widespread geographically of the arboviruses and a major public health threat in the tropics and subtropics. Whereas malaria is declining in many parts of the world, dengue infections continue to rise in numbers and to expand geographically. Effective vector control remains elusive. The pathomechanism for severe disease remains poorly understood. Lack of animal models has impeded scientific advances. Anti-dengue compounds have been identified but none have shown clinical promises to date. The recent setback in the first efficacy results with the chimeric dengue vaccine indicate that the development of a dengue vaccine is more challenging than anticipated. All these problems highlight that dengue is a hard nut to crack.

Giardiasis

Christoph F.R. Hatz, MD, DTM&H

Professor of Tropical and Travel Medicine, Swiss Tropical and Public Health Institute, Basel, Switzerland; and University of Basel, Basel, Switzerland. Professor of Epidemiology and Prevention of Communicable Diseases and Director of World Health Collaborating Center, Division of Communicable Diseases, Institute of Social and Preventive Medicine, University of Zuerich, Switzerland

Giardia lamblia is the most common intestinal protozoan infection of man. Its prevalence depends on the level of hygiene: ~ 1000 million cases at any one time, highest prevalence in slums of Asia, Africa, Latin America, mainly in children (up to 80 %). In Central Europe it is estimated to be at < 1%. Among travellers, it is the most common intestinal protozoan infection, and the most common cause of chronic diarrhoea. Most cases of giardiasis are due to anthroponotic spread. Zoonotic transmission is possible, but rare. The cyst of *G. lamblia* measures 8–15 µm × 7–10 µm, the one of trophozoites 9–20 µm x 5–12µm. Depending on the destination of the travellers, the parasite's prevalence accounts for up to 5% of travellers' diarrhoea cases. Clinical signs include acute and chronic diarrhoea, flatulence and bloating, foul-smelling greasy stools (often light or yellow colour), malaise and abdominal cramps. Fever is not a typical feature. Incubation period is between 3 days and 3 weeks. After natural infection, 50% develop symptomatic disease, 15% are asymptomatic excretion of cysts, and 35% have no sign of infection. Due to the disruption of mucosal architecture (flattening of villi) of the gut, epithelial disacharidases are lost, explaining the frequently observed secondary lactose intolerance.
Diagnosis is made with a variety of approaches: stool microscopy (1 sample yields 50-70%; 3 samples yield >90%), or by rapid antigen detection (ELISA) in stool (sensitivity: antigen detection > microscopy). Duodenal aspirate, the "string test", duodenal biopsy using endoscopy, as well as empirical treatment are recommended and/or practised by some experts. Management of *G. lamblia*: a study among 53 centres of the European network TropNet revealed 39 different regimens using 7 drugs alone or in combination in different dosage & duration. 5-Nitroimidazoles are the most commonly used drugs against *G. lamblia* worldwide, followed by benzimidazoles. Nitazoxanide, a 5-nitrothiazolyl derivate, is being used more often now. The uncertainty of which drugs to use for first treatment and in treatment failure is disputed and requires more studies.

11:15 - 13:00 **Session I: Travel Medicine**

Chair: Michal Chowers & Michael Alkan

11:15 – 11:40 **Vaccine preventable encephalitis in travelers: Rabies and Japanese encephalitis vaccine** - Christoph F.R. Hatz (Switzerland)

11:40 – 12:00 **Meningococcal vaccines: Indications and strategies in travelers** - Annelies Wilder-Smith

12:00 – 12:15 **High incidence of food borne illness among travelers to Armenia** - Drorit Attias & Eli Schwartz

12:15 – 12:30 **Laboratory characteristics of non-immune malaria patients in Israel** - Shlomit Keller, Eyal Leshem, Nathan Keller & Eli Schwartz

12:30 – 12:45 **Possible risks of travel-related medications in a cohort of Israeli travelers to tropical countries** - Shmuel Stienlauf, Bianca Streltsin, Eran Kopel, Eyal Leshem, Eyal Meltzer, Daniel Kurnik, Gad Segal, Shaye Kivity and Eli Schwartz

12:45 – 13:00 **Re-emergence of pertussis and introduction of pertussis vaccine to travelers** - Esther Marva, Lea Valinsky, Dana Mizrahi, Vered Agmon, Emilia Anis, Paul Slater, Ruslan Gusinov, Daniele Goldmann & Larisa Moerman

Vaccine preventable encephalitis in travelers: Rabies and Japanese encephalitis vaccine

Christoph F.R. Hatz, MD, DTM&H

Professor of Tropical and Travel Medicine, Swiss Tropical and Public Health Institute, Basel, Switzerland; and University of Basel, Basel, Switzerland; Professor of Epidemiology and Prevention of Communicable Diseases and Director of World Health Collaborating Center, Division of Communicable Diseases, Institute of Social and Preventive Medicine, University of Zurich, Switzerland

Rabies is a zoonotic viral disease, transmitted only in mammals, mostly *Carnivora* and *Chiroptera*. The genus Lyssavirus contains several rabies rabies-related viruses (see Table). Transmission occurs by the virus entering through the skin or the mucosa after bites or scratches by an infected mammal. More than 99% of human deaths are caused by dog bites.

Table. Rabies and rabies-related viruses.

Geno type	Virus	Phylogroup	Identified in	Main host
1	Rabies Virus (RABV)	I	Worldwide	canine, bat
2	Lagos (LBV)	II	Africa, Middle East	bat
3	Mokola	II	West Africa (Nigeria)	bat
4	Duvenhage	I	Africa	bat
5	EBLV-1	I	Europe	bat
6	EBLV-2	I	Europe	bat
7	Australian bat (ABLV)	I	Australia	bat
8	Aravan (ARAV)	I	Kyrghystan	bat
9	Khujand (KHUV)	I	Tajikistan	bat
10	Irkut	I	Asia	bat
11	West Caucasian bat virus	?	Eastern Europe	bat
	Shimoni bat virus* (SHIBV)	II	Kenya	bat
	Bokeloh bat virus* (BBLV)	I	Germany	bat

*Not definitively classified

Prevention of rabies in travellers: The incidence of being licked or bitten by a potentially rabid animal (mostly dogs) among travellers is estimated at a maximum of 3.6%. Management after potential rabid contact: Immediate thorough (approx. 15 min) washing and flushing of all bite wounds and scratches with alkaline soap and great amounts of water, followed by post-exposure prophylaxis with vaccines (Intramuscular regimens: 1 dose on days 0, 3, 7, 14, 28 together with immunoglobulins, or Zagreb regimen: 2 doses on day 0, followed by 1 dose on day 7 and 21 each in case that no immunoglobulin is available. Intradermal regimens: two intradermal doses on days 0, 3, 7 and 28).

Japanese encephalitis (JE), caused by a zoonotic flavivirus and transmitted by mosquitos during twilight and at night, is the most common vaccine-preventable viral encephalitis in rural areas from Eastern Pakistan to Northern Queensland in Australia and to Japan. The JE risks for two destinations with the highest risk for short-term travellers, Bali in Indonesia and Thailand, are estimated to be one case in 1 and 3.3 million travellers. The fact that a new, apparently safe vaccine is now available is not necessarily a reason to boost its use in a population that may be at very limited risk.

Meningococcal vaccines: Indications and strategies in travelers

Annelies Wilder-Smith

National University of Singapore

Meningococcal disease patterns and incidence can vary dramatically, both geographically and over time in populations, influenced by differences in invasive meningococcal capsular serogroups and specific genotypes designated as ST clonal complexes. Serogroups B, C, and Y are responsible for the majority of cases in Europe, the Americas, and Oceania; serogroup A has been associated with the highest incidence (up to 1000 per 100,000 cases) and large outbreaks of meningococcal disease in sub-Saharan Africa and previously Asia; and serogroups W-135 and X have emerged to cause major disease outbreaks in sub-Saharan Africa. Significant declines in meningococcal disease have occurred in the last decade in many developed countries, mainly due to the introduction of new meningococcal vaccines. Serogroup C polysaccharide-protein conjugate vaccines were introduced over a decade ago, first in the UK in a mass vaccination campaign, and are now widely used; multivalent meningococcal conjugate vaccines containing serogroups A, C, W-135, and/or Y were first used for adolescents in the US in 2005 and have now expanded indications for infants and young children, and a new serogroup A conjugate vaccine has recently been introduced in sub-Saharan Africa. The effectiveness of these conjugate vaccines has been enhanced by the prevention of person-to-person transmission and herd immunity. Risk of meningococcal disease in travelers and vaccine strategies will be discussed.

High incidence of food borne illness among travelers to Armenia

Drorit Attias & Eli Schwartz

The Center of Geographic Medicine, Sheba Medical Center, Tel Hashomer and the Medical Center for the Traveler, Jerusalem, Israel

Introduction: Lately, Armenia has become an important traveling destination for Israeli travelers. Food and water borne infections are the most important health issue among international travelers**,** but yet there is limited information about the incidence of these infections in Armenia. Tap water and spring water in Armenia are considered to be safe by several travel-related authorities.

Methods: A prospective observational study about symptomatic gastro-intestinal infections in women traveled to Armenia was conducted. We followed 240 women in 6 - consecutive jeep-expeditions of 10 days each to Armenia. The journeys were conducted from June to August 2012. Preventive strategies such as hygiene measures were given by each physician accompanying the journey. Symptoms were recorded during the trip and the travelers were followed for another 1 month after the trip.

Results: Altogether 62/240 (26%) participants had symptoms of food borne illness during and after the journeys. 42 women had diarrhea and/or vomiting for 1-3 days during the trip. Post-travel symptoms occurred in 20 women. Symptoms included abdominal pain, diarrhea, nausea and also extra-intestinal manifestations such as severe fatigue, headaches and arthralgia. 14/20 women had spontaneous cure after 7-10 days, while 6/ 20 (30%) out of this group recovered after empiric anti-parasitic treatment with Tinidazole (Protocide®) despite negative stool for parasite. Symptoms of 3 patients lasted up to 8 weeks because of misdiagnosis and lack of proper treatment. Incidence rate is 36% per 2 weeks stay.

Conclusion: The attack rate of 26% during these 10 days trip is equivalent to an incidence rate of 36% per 2 weeks stay, indicating that Armenia should be considered as country with high risk for contracting food borne infections. Travelers to this area should be made-aware of this risk by travel medicine practitioners. It is advised to give empiric therapy with anti-parasitic medications, such as Tinidazole, to travelers returning with prolonged symptoms of abdominal pain, diarrhea and/or fatigue.

Laboratory characteristics of non-immune malaria patients in Israel

Shlomit Keller, Eyal Leshem, Nathan Keller & Eli Schwartz

Sheba Medical Center, Tel Hashomer

Human malaria is a main cause of morbidity and mortality in returning travelers from endemic countries. Symptoms of the disease in its early stages can simulate common febrile diseases, thus without high index of suspicion, the physician can easily misdiagnose the disease. Both clinical and laboratory characteristics of malaria in travelers (a non-immune population) differs from characteristics of malaria in a population living in endemic areas that are exposed to the disease throughout their lives (immune population).

Objectives – Our aim was to characterize routine laboratory results of non-immune (Israeli) travelers suffering from imported malaria of all species following return from endemic areas.

Methods - A retrospective analysis of malaria patients' laboratory data. Data were collected from records of patients diagnosed and hospitalized at Sheba Medical Center, between the years 2004- 2011. Patient records were retrieved from the database of the Center of Travel Medicine, Clinical Microbiology, and the general Laboratory at Sheba Medical Center. In order to include only a non-immune population we excluded patients born in malaria endemic areas. Thus, the study population included only travelers who are originally from Israel or from other Western countries (defined as non-immune populations). For each patient, the demographic data, type of journey and laboratory tests at admission and during hospitalization were collected. We also compared the laboratory tests at admission of *P. falciparum* patients with *P. vivax* patients.

Results – During this period of 7 years there were 135 patients diagnosed with malaria at the Sheba Medical Center. Of these patients, 83 were eligible. Reasons for travel included business 50/83 (60%), and travel / tourism 33/83 (40%). Most cases were caused by *P. falciparum* 46/83 (55%) and *P. vivax* 31/83 (37.3%). The rest were *P. ovale* (4/83) and *P. malariae* (2/83). Pertinent laboratory findings on admission included anemia in 11/83 (13%), leukopenia 21/83 (25%), and thrombocytopenia in 61/83 (73.5%) patients. Increased LDH was found in 69/83 (83%) of patients, increased bilirubin in 45/83 (50%), increased liver enzymes (AST and ALT) were found in 21/83 (25%) of patients. Increased creatinine was found in 30/83 (26.5%) of patients. In addition to the routine tests, 62/67 (92.5%) showed low total cholesterol values (<200 mg/d). D-dimer values were increased in 44/63 (70%) of patients, with a mean of 1737 ± 2658.28 ng/dl. Blood count (CBC) on admission was within normal limits in 17 patients (20.5%). Chemistry tests were within normal limits in 8 patients (10%). Both CBC and chemistry were within normal limits in 5 patients (6%), and in 8 patients (10%) with one minimal exception value (range of up to 15% of normal). Comparing P. *falciparum* to *P. vivax* infection showed that in patients with *P. falciparum* increased values of D-Dimer (2307.05 ± 3073.3 vs. 718.72 ± 1436.87, $P<0.05$), AST (55.76 ± 41.64 vs. 30.23 ± 18.76, $P<0.05$), ALT (65.4 ± 76.14 vs. 36.61 ± 28.21, $P<0.05$), LDH (417.37 ± 220.71 vs. 268.87 ± 86.82, $P<0.05$) and creatinine (1.21 ± 0.34 vs. 1.05 ± 0.22, $P<0.05$) were observed.

Possible risks of travel-related medications in a cohort of Israeli travelers to tropical countries

Shmuel Stienlauf, Bianca Streltsin, Eran Kopel, Eyal Leshem, Eyal Meltzer, Daniel Kurnik, Gad Segal, Shaye Kivity and Eli Schwartz

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Background: Data regarding the frequency of possible interactions between travel-related medications (TRM) and chronic medications used by travelers to developing countries is limited. In addition, caution is advised in the use of some TRM in several medical conditions. We sought to calculate the frequency of TRM interactions and precautions.

Methods: A retrospective cohort study of travelers who attended the Sheba Medical Center Travel Clinic between the years 2005-2007. We analyzed demographics, travel destinations, chronic use of medications and drug allergies among travelers. The following TRM were evaluated: fluoroquinolones, rifaximin, azithromycin, atovaquone/proguanil, mefloquine, primaquine and acetazolamide. Drug interactions of TRM were retrieved from the Micromedex® Healthcare Series.

Results: A total of 16,681 travelers attended the pre-travel clinic during 3- year period. Of those, 2,221 (13%) travelers reported chronic medication use. Seventeen hundred possible drug interactions between at least one TRM and chronic medications were found in 780 travelers (35% of travelers taking chronic medications, 5% of all travelers). The following table summarizes the frequency of interactions in TRM:

TRM	**Travelers Taking Chronic Medications with possible interaction Number (%)**	**Total number of Interactions (n)***
Fluoroquinolone	654 (29)	752
Azithromycin	598 (27)	625
Mefloquine	152 (7)	157
Acetazolamide	117 (5)	118
Atovaquone/proguanil	24 (1)	24
Rifaximin	23 (1)	23

*Travelers may take more than 1 drug with potential interaction

Primaquine use as malaria prophylaxis was contraindicated in 204 travelers (1.2%) because of glucose-6-phosphate dehydrogenase deficiency. Sulfonamide allergy was found in 100 travelers (0.5%), which may pose a relative contraindication for the use of acetazolamide. Similarly, caution regarding fluoroquinolone use was advised in 52 travelers (0.3%) which had central nervous system disorders.

Conclusions: Interactions between TRM and medical conditions or chronic medications occur in a significant proportion of travelers. Fluoroquinolone antibiotics and azithromycin have the largest potential for drug interactions among TRM. These data should be taken into account by the travel health practitioner consulting travelers to developing countries.

Re-emergence of pertussis and introduction of pertussis vaccine to travelers

Esther Marva[1], Lea Valinsky[1], Dana Mizrahi[1], Vered Agmon[1], Emilia Anis[2], Paul Slater[2], Ruslan Gusinov[2], Daniele Goldmann[2] & Larisa Moerman[2]

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Background: Pertussis is a vaccine-preventable disease that has re-emerged in Israel and other industrial counties. The reported crude incidence of the disease increased 22-fold since 1998. To decrease the pertussis morbidity two booster doses were introduced in 2005 and 2008 to school aged children and from 2011 pertussis vaccine was recommended to traveler.
Objectives: To describe the epidemiology of pertussis and to explain the substantial increase in reported pertussis incidence in Israel in recent years.
Methods: Crude and specific pertussis incidence by age, patient immunization status, hospitalization rate, and national immunization coverage rate were calculated from reports provided by the Public District Health Offices of the Ministry of Health.
Results: National pertussis immunization coverage by age 2 years was stable during the last 10 years. Nevertheless, the reported crude incidence of pertussis increased from 1.4/100,000 in 1998 to 30.7/100,000 in 2011. The trend was observed in all age groups, being most prominent in infants under age 1 year and in children aged 5–14. The incidence of pertussis was substantially higher in unvaccinated and partly vaccinated compared to fully vaccinated persons. Hospitalization rate in infants under age of 1 year was much higher than in other age groups in 2005 – 2011 years.
Conclusions: There are several possible explanations for the re-emergence of pertussis: waning of vaccine-induced immunity, increased physician awareness and reporting and the availability of more sensitive diagnostic methods (serology and RT-PCR) and genetic divergence of circulating pertussis strain from vaccine strains. In addition to providing two booster doses to school age children, we now offer Tdap to travellers who require tetanus and diphtheria booster, in order to increase population immunization to pertussis.

11:15 - 13:00 **Session 2: Molecular Parasitology**

Chairs: Dan Zilberstein & Michal Shapira

11:15 – 11:30 **Signalling for *Leishmania* differentiation induces a parasite-specific protein kinase pathway** - Polina Tsigankov, Pier Federico Gherardini, Manuela Helmer-Citterich & Dan Zilberstein

11:30 – 11:45 **Functional genomics of amino acid transporters in the human pathogen *Leishmania*: the story of proline and alanine** - Dan Zilberstein, Ehud Inbar, Doreen Schlisselberg, Marianne Suter Grotemeyer & Doris Rentsch

11:45 – 12:00 **New and old players in the translation apparatus of *Leishmania* - the role of cap-binding proteins and the eIF3 complex in adapting to physiological stresses** - Alexandra Zinoviev, Shahar Manor and Michal Shapira

12:00 – 12:15 **The role of cis element and antisense ncRNAs in var gene regulation in the malaria parasite *Plasmodium falciparum*.**- Inbar Avraham & Ron Dzikowski

12:15 – 12:30 **A novel Plasmodium falciparum SR protein is an alternative splicing factor that is required for parasite proliferation in human erythrocytes** - Eshar S., Alemand E., Sebag A., Glaser F., Mandel-Gutfreund Yael, Karni R. & R. Dzikowski

12:30 – 12:45 **Identification of *S*-nitrosylated proteins in *Entamoeba histolytica* with emphasis on Dnmt2 regulation by Nitric Oxide** - Rivi Hertz & Serge Ankri

12:45 – 13:00 **Evaluation of quantitative real-time kinetoplast DNA-PCR for detecting and quantifying *Leishmania donovani* in large numbers of dried human blood samples -from a visceral leishmaniasis focus in Northern Ethiopia** - Samar Aramin, Ibrahim Abassi, Asrat Hailu & Alon Warburg

Signalling for *Leishmania* differentiation induces a parasite-specific protein kinase pathway

Polina Tsigankov[1], Pier Federico Gherardini[2], Manuela Helmer-Citterich[2] and Dan Zilberstein[1]
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During infection, the extracellular insect forms (promastigotes) of *Leishmania* parasites undergo rapid differentiation to intracellular amastigotes that proliferate in phagolysosomes of mammalian macrophages. This process is simulated *in vitro* by shifting cultured promastigotes (grown at 26ºC, pH 7) to a lysosome-like environment (37ºC and pH 5.5, differentiation signal). Time course analyses showed that promastigotes differentiation into amastigotes is a regulated process that involves programmed changes in morphology, protein and gene expression. The aim of this study was to identify the signaling pathway that initiates *Leishmania* differentiation. First, we determined changes in *Leishmania donovani* phosphoproteome during differentiation, and then looked for signal-specific protein kinase activities at the beginning of differentiation. Our analysis revealed a number of changes in protein phosphorylation during differentiation; Phases I (signal perception) and III (morphology change) were associated with more protein phosphorylation, while phases II (movement cessation) and IV (amastigote maturation) showed greater de-phosphorylation. Differentiation signal employed distinct trends in proteins with multiple phosphorylation sites as well as dynamic changes in protein phosphorylation. Our analysis revealed novel protein kinases that are phosphorylated specifically upon differentiation signal at the beginning of phase I, but not when exposed to either high temperature or acidic pH alone. We hypothesize that these protein kinases initiate the pathway that trigger promastigotes differentiation into amastigotes.

Functional genomics of amino acid transporters in the human pathogen *Leishmania*: the story of proline and alanine

Dan Zilberstein[1], Ehud Inbar[1], Doreen Schlisselberg[1], Marianne Suter Grotemeyer[2] and Doris Rentsch[2]

[1]Faculty of Biology, Technion-Israel Institute of Technology, Haifa 32000, Haifa, Israel and [2]Institute of Plant Sciences, University of Bern, 3013 Bern, Switzerland

Unlike all other organisms, *Leishmania* (as well as all members of the Trypanosomatid family) maintain a large cellular pool of proline that, together with alanine, serves as an alternative carbon source and as a reservoir of organic osmolytes. We cloned and characterized a new neutral amino acid transporter, LdAAP24 that translocates proline and alanine across the *L. donovani* plasma membrane. By knocking out the gene that encodes for *LdAAP24* we showed that this transporter fulfills multiple functions: it is the sole supplier for the intracellular pool of proline and contributes to the alanine pool; it is essential for cell volume regulation after osmotic stress; and it regulates transport and homeostasis of glutamate, arginine and glycine, none of which are its substrates. Interestingly, loss of cellular proline signal for parasites in the insect to develop virulence, a process called metacyclogenesis; Δ*ldaap24* mutants transiently express gene markers of metacyclogenesis, at late log rather than late stationary phase. In addition, they change to metacyclic-like morphology at mid log, but resume oval shape at stationary phase. Finally, Δ*ldaap24* are significantly more susceptible to oxidative stress than wild type. We conclude that by controlling the cellular pool of proline LdAAP24 plays a key role in virulence development of *Leishmania* parasites inside the vector.

New and old players in the translation apparatus of *Leishmania* - the role of cap-binding proteins and the eIF3 complex in adapting to physiological stresses

Alexandra Zinoviev, Shahar Manor and Michal Shapira

Department of Life Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel

Many eukaryotes encode multiple isoforms of the cap-binding translation initiation factor (eIF4E).*Leishmania* and trypanosomatids encode four paralogs of this protein, but none can complement the eIF4E function in a yeast mutant. A low conservation is observed between the four paralogs, suggesting they assist these organisms survive a multitude of conditions encountered throughout the life cycle. Earlier attempts to decipher their function led to identification of LeishIF4E-4 as the traditional translation initiation factor. LeishIF4E-1 appears to function during thermal stress, via a mechanism not yet understood. LeishIF4E-3 hardly binds cap-4 and thus cannot serve as a typical initiation factor. Although it interacts with an eIF4G homolog, LeishIF4G-4, the two polypeptides do not co-migrate on sucrose gradients. While LeishIF4E-3 enters large particles that increase in size during nutritional stress, LeishIF4G-4 is found only in the top fractions, lighter than the 43S pre-initiation complex. Confocal microscopy localized LeishIF4E-3 (but not LeishIF4G-4) within nutritional stress-induced granules. Accordingly, interaction between the two proteins reduced upon starvation. We therefore propose that under normal conditions, LeishIF4G-4 sequesters LeishIF4E-3 in the cytoplasm. During a nutritional stress, LeishIF4E-3 is modified and released from LeishIF4G-4 to enter stress granules, where inactive mRNAs are stored. Structural aspects of the interaction between LeishIF4E-3 and LeishIF4G-4 will be discussed, as these are non- conserved and do not follow the canonical rules for such interactions, as known from higher eukaryotes. These data make LeishIF4E-3 an interesting pharamacological target.

The role of cis element and antisense ncRNAs in var gene regulation in the malaria parasite *Plasmodium falciparum*.

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Plasmodium falciparum parasite causes the deadliest form of human malaria. The parasite genome contains ~60 *var* genes. Each individual parasite expresses only a single *var* gene at a time, maintaining the remaining *var* genes in a transcriptionally silent state. All *var* genes share a similar structure: a *var* promoter upstream of a open reading frame and two exons seperated by a well conserved intron. Within the intron ther is a bidirectional promoter that gives rise to two sterile transcripts with unknown function in both sense and antisense direction (noncoding RNAs). Strict pairing between the *var* promoter and the intron is required for both silencing and counting of individual *var* gene. This suggests that each individual *var* gene contains the cis regulatory elements that enable it to be properly regulated. We transfected clonal populations of parasites that exclusively express a known endougenous *var* gene with en episome that mimics a *var* gene structure. This plasmid is by default silent, suggesting that this construct that contains both *var* promoter and intron is properly regulated. Interestingly, by modifying this plasmid in different ways, we were able to interfere with the strict pairing between the *var* promoter and the intron and to achieve constitutively active episome that is not counted by the mechanism that controls mutually exclusive expression. We have identified a DNA element at the *var* Intron and the *var* promoter that acts as an insulator and is required for both silencing and mutually exclusive expression of *var* genes [Fig.1]. We also demonstrated by Electro Mobility Shift Assay that this motif forms a protein-DNA complex and serves as a target for specific proteins. Additionally, we measured the antisense ncRNA levels in active and silenced endogenous genes and demonstrated that the antisense sterile transcript of the intron promoter is associated with active *var* gene. We over expressed a specific *var* antisense ncRNA on an episome and showed a direct influence upon activation of the endogenous *var* gene.

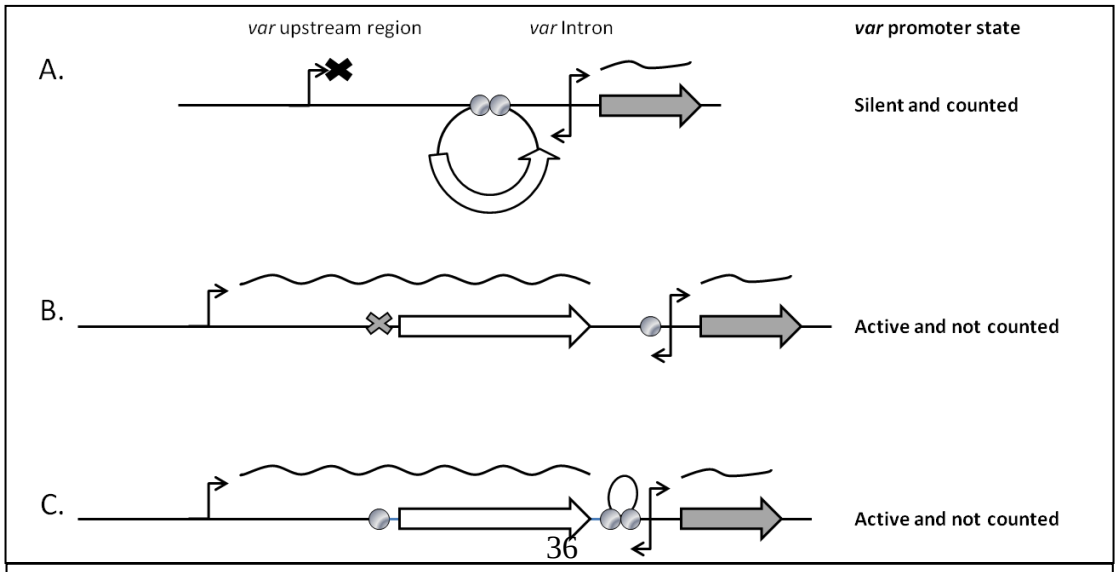

Figure 1: DNA elements, functions as Insulators, are important for *var* gene silencing. A model suggesting that DNA motifs found within *var* promoter and intron, mediates a proper interaction between them and responsible for *var* gene silencing.

A novel *Plasmodium falciparum* SR protein is an alternative splicing factor that is required for parasite proliferation in human erythrocytes

Eshar S.[1], Alemand E.[2], Sebag A.[3], Glaser F.[4], Mandel-Gutfreund Yael[4], Karni R.[3], Dzikowski R.[1]

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The malaria parasites have a complex life cycle, during which it undergoes significant biological changes to adapt to different hosts and changing environments. *Plasmodium falciparum*, the deadliest form of human malaria, has adapted to its complex life cycle with relatively small number of genes. Alternative splicing (AS) is an important post-transcriptional mechanisms that enables eukaryotic organisms to expand their protein repertoire out of relatively small number of genes. SR proteins are major regulators of splicing in higher eukaryotes. Nevertheless, the splicing as well as the AS machinery in *Plasmodium spp*. are still elusive. We show that PfSR1 is an SR protein that can mediate RNA splicing *in vitro*. In addition, we demonstrate that PfSR1 functions as an alternative splicing factor in a mini-gene system similar to the mammalian SRSF1. Expression of PfSR1-*myc* in *P. falciparum* shows distinct patterns of cellular localization during intra erythrocytic development. Furthermore, we determine that the predicted RS domain of PfSR1 is essential for its localization to the nucleus. Finally, we demonstrate that proper regulation of *pfsr1* is required for parasite proliferation in human RBCs, and affect the splicing pattern of endogenous genes.

Identification of *S*-nitrosylated proteins in *Entamoeba histolytica* with emphasis on Dnmt2 regulation by Nitric Oxide

Rivi Hertz and Serge Ankri

Department of Molecular Microbiology, The Bruce Rappaport Faculty of Medicine, Technion.

Protein S-nitrosylation has emerged as the main mechanism of nitric oxide (NO)-based signalling and a fundamental component of redox-based physiological regulation. Evidence is now emerging that NO is also a regulator of epigenetic events because it can modify components of the epigenetic machinery. During its life cycle, *Entamoeba histolytica* must adapt to various environmental stresses during infection of their human hosts, and the host-parasite relationship is dependent upon the host's immune system. NO is also the major cytotoxic molecule that is released by activated macrophages, natural killer cells, and other phagocytic cells for killing *E. histolytica* trophozoites. It has also been recently reported that NO triggers stress responses in *E. histolytica* and that NO directly inhibits glycolysis and stimulates cysteine synthase activity. Despite these reports on the actions of NO on *E. histolytica* trophozoites, our knowledge on the S-nitrosylation of *E. histolytica* proteins is lacking. Moreover, identification of those *E. histolytica* proteins that are susceptible to *S*-nitrosylation would help us improve our current understanding of S-nitrosylation in *E. histolytica* physiology. In order to determine whether *S*-nitrosylation of proteins of the parasite, *Entamoeba histolytica* regulates their function, we used the resin-assisted capture (SNO-RAC) method to identify *S*-nitrosylated proteins. We observed that SNO proteins of *E. histolytica* are mainly involved in glycolysis/gluconeogenesis, pyruvate metabolism, and ribosome synthesis. The *E. histolytica* Dnmt2 (Ehmeth) and its inhibitor, enolase, were also found among the SNO proteins. Interestingly, we found that NO reduces the formation of Ehmeth-enolase complex leading to an overall increase in $tRNA^{Asp}$ and DNA methylation. We also found that GSNO stimulates the tRNA methyltransferase activity of Dnmt2. Finally, we report that Ehmeth protects the parasite against nitrosative stress. In summary, the results of this investigation provide the first global analysis of SNO proteins in *E. histolytica* and the first evidence that *S*-nitrosylation of proteins, such as Dnmt2, is a regulator of the parasite's epigenetic machinery.

Evaluation of quantitative real-time kinetoplast DNA-PCR for detecting and quantifying *Leishmania donovani* in large numbers of dried human blood samples from a visceral leishmaniasis focus in Northern Ethiopia.

Samar Aramin[1], Ibrahim Abassi[1], Asrat Hailu[2] & Alon Warburg[1]

[1]Department of Microbiology and Molecular Genetics, IMRIC, The Kuvin Centre for the Study of Infectious and Tropical Diseases, Hadassah Medical School, The Hebrew University of Jerusalem, 91120, Israel; [2]Faculty of Medicine, Addis Ababa University, P.O.Box 9086, Addis Ababa, Ethiopia

Background

Visceral leishmaniasis (VL) known as Kala-Azar, is a systemic protozoan infection caused by intracellular parasites named *Leishmania donovani*. Worldwide, an estimated 500,000 VL cases occur annually, over 90% of which are concentrated in the Indian sub-continent, East Africa and Brazil. In Africa, the worst affected regions are in southern Sudan (15,000-20,000 cases) and Ethiopia with 4,000-5,000 diagnosed VL cases a year, and 4.0 million people living in endemic foci. PCR-based methods for detecting parasites are highly sensitive and may be performed on dry specimens without the need for cold-storage. We assessed the efficacy of quantitative real-time kinetoplast DNA/PCR (qRT-kDNA PCR) for detecting and quantifying *L. donovani* DNA in dried blood.

Methods:

Some 5,000 volunteer villagers living in the endemic area of Sheraro in Northern Ethiopia, were selected for a cohort study aimed at elucidating the role of symptomatic and asymptomatic *Leishmania donovani*-infected persons in the epidemiology of VL. Dried-blood samples were tested by qRT-kDNA PCR to detect and quantify *Leishmania* kinetoplast DNA.

Results:

Of the ~4757 dried-blood samples tested, 680 (14.3%) were found positive for *Leishmania* DNA, but most of these (69%) had less than 10 parasite/ml of blood. To validate the results, we reexamined some of the samples using the same methodology. Only 59.3% of the samples estimated to harbor less than10 parasite/ml, were positive the second time. Furthermore, 10.8% of the samples that were qRT-kDNA PCR negative in the initial screening, were positive during the second test. Almost all samples with higher parasitemias remained positive upon re-examination. To determine the *Leishmania* species, ITS1 PCR products from 22 samples were sent for sequencing. Results showed that 20/22 samples were *L. donovani* while two had ITS1 sequences homologous to *L. major*. We also compared different methods for DNA preparation. DNA extraction using the phenol/chloroform method was more efficient than either sodium hydroxide or potassium acetate.

Conclusions:

Although qRT-kDNA PCR is a highly sensitive test, the dependability of low positives remains questionable. The infectiousness of different parasitemias for vector sand flies needs to be determined by xenodiagnosis. Additional parameters such as anti Leishmania antibodies and skin tests should also be performed.

14:00 – 16:00	**Session 3: Tropical Medicine**
	Chairs: Nathan Keller & Mervyn Shapiro
14:00 – 14:15	**NS1 antigen testing for the diagnosis of Dengue in returned Israeli travelers -** Inbal Fuchs, Hana Bin, Sara Schlesinger & Eli Schwartz
14:15 – 14:30	**Acute hepatitis in Israeli travelers -** Tamar Lachish & Eli Schwartz
14:30 – 14:45	**Recurrent furunculosis in returning travelers -** Artzi, Ofir, Sinai, Maya & Schwartz, Eli
14:45 – 15:00	**Case report: Thailand- Not just a pretty place – Clinical case and review of the literature -** Michal Katzir & Michal Chowers
15:00 – 15:15	**Case report: Not always what it looks like – A refugee from Eritrea with a tumor-like mass in the liver -** Yael Paran & Steve Berger
15:15 – 15:30	**The changing epidemiology of human African tryponasomiasis among patients from non-endemic countries: 1902-2012 –** Ami Neuberger, Eyal Meltzer, Eyal Leshem, Yaakov Dickstein, Shmuel Steinlauf & Eli Schwartz
15:30 – 15:45	**"Tropical" and unusual infections among undocumented migrants who are treated at the designated clinics in Tel-Aviv, Israel -** Zohar Mor, Nadav Davidovitch, Ido Lurie, Alex Leventhal, Michael Dor & Itamar Grotto
15:45 – 16:00	**Discussion**

NS1 antigen testing for the diagnosis of Dengue in returned Israeli travelers

Inbal Fuchs MD[1], Hana Bin PhD[2], Sara Schlesinger[2], Eli Schwartz MD, DTMH[3]

The Pediatric Infectious Disease Unit, Soroka University Medical Center, Ben-Gurion University, Beer-Sheva[1], National Center for Zoonotic Viruses, Central Virology Laboratory, Ministry of Health, Public Health Services, Sheba Medical Center, Tel Hashomer. [2], Center for Geographic Medicine and Tropical Diseases Sheba Medical Center, Tel Hashomer, Israel [3]

Background: Acute disease due to Dengue virus infection is a common cause of illness in Israeli travellers. Antigen testing with NS1 provides rapid diagnosis during the febrile phase of illness before appearance of IgM in serum in patients from Dengue-endemic areas.

Aim: We aimed to determine the diagnostic accuracy of NS1 antigen testing in travellers returning from Dengue endemic countries with clinical illness and serologically confirmed Dengue infection. Sera of patients with serologically confirmed West Nile virus disease were used as controls.

Methods: Sera obtained from patients who returned from Dengue-endemic countries with positive Dengue-IgM and sera from patients with confirmed West Nile virus infection were tested for NS1 antigen using the Panbio Dengue Early ELISA assay within 21 days of symptom onset. Demographic data and travel destination as well interval between testing and disease onset were retrieved retrospectively from patient files. Sensitivity and specificity were calculated as a function of time since symptom onset.

Results: Fifty eight sera from 40 Dengue- infected patients and 26 sera from 26 West Nile virus- infected patients were tested. Sensitivity of NS1 testing in Dengue patients was 87% during the first 3 days of symptoms and declined to about 70% during days 4-7. Specificity was 92% for the entire testing period.

Conclusions: The NS1 Panbio assay is sensitive for the detection of DEN viral infection in returning travellers during the febrile phase of illness, and is specific for DEN in a region where West Nile virus co-circulates.

Acute hepatitis in Israeli travelers

Tamar Lachish[1], Eli Schwartz[2]

[1]The Infectious Diseases Unit, Shaare-Zedek Medical Center, Jerusalem, Israel. [2]The Center for Geographic Medicine and Tropical Diseases, the Chaim Sheba Medical Center, Tel Hashomer & Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Background – Acute hepatitis is a well described cause of morbidity and sporadic mortality in travelers. Data regarding the epidemiology of hepatitis in travelers are lacking. The aim of this study is to describe the epidemiology of acute viral hepatitis among travelers returning from tropical countries, with particular attention to enetrically-transmitted hepatitis.

Methods – This study is a prospective observational study of ill-returned travelers who presented at two travel medicine clinics in Israel between the years 1997 to 2012. Data of patients with acute hepatitis were summarized. Only travelers were included, immigrants and foreign workers were excluded.

Results – Among 4970 Israeli travelers who were seen during this period, 49 (1 %) were diagnosed with acute hepatitis. Among them, hepatitis E virus (HEV) was the etiology in 19 (39%) cases and Hepatitis A virus (HAV) was the etiology in 13(27%) cases, demonstrating that 65 % of all cases were due to enterically-transmited hepatitis Acquiring acute hepatitis B (2 cases) or acute hepatitis C (1 case) were uncommon (6.1%). In 27% percent of the cases, no diagnosis was determined. Fifty five percent of all cases were imported from the Indian subcontinent, with a predominance of HEV infection (84%). A significant male predominance was seen in all groups regardless of etiology.Pretravel consultation was documented in only 7% of those with vaccine preventable hepatitis (hepatitis A &B) compared to 89% in those with Hepatitis E.

Conclusion – Enterically-transmited hepatitis is the main causes of viral hepatitis among travelers. Hepatitis E virus is an emerging disease and has become the most common hepatitis among Israeli travelers. Although an efficacious vaccine has been developed, no licensed HEV vaccine is yet available. Although hepatitis A vaccine is highly efficacious, safe and easily available, there is a steady prevalence of HAV cases. Further follow-up is needed to determine whether the Israeli national program for HAV vaccination in infancy will affect the epidemiology of hepatitis among travelers.

Recurrent furunculosis in returning travelers

Artzi, Ofir, Sinai, Maya & Schwartz, Eli

Department of Dermatology and Center for Geographic Medicine and Tropical Diseases, Chaim Sheba Medical Center, Tel Hashomer, Israel and Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

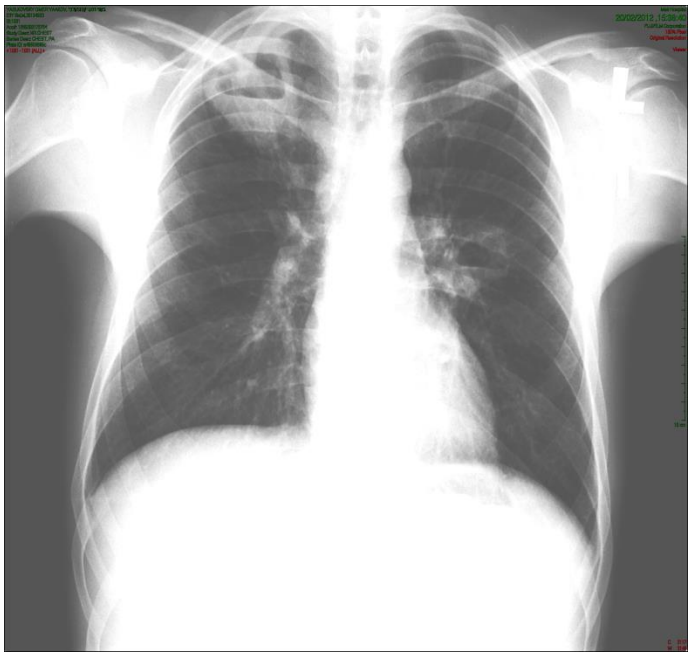
Dermatologic conditions are common among returning travelers and account for 18% of all doctor visits of ill returning travelers. Skin abscess or furunculosis is the third most common dermatologic diagnosis in travelers (after Cutaneous larvae migrans and insect bite) and usually resolves spontaneously or with antibiotics, without sequela. This retrospective study describes a group of young, healthy individuals, who presented to the tropical disease clinic of Sheba Medical Center with recurrent furunculosis after returning from a trip to a tropical region. To our knowledge, recurrent furunculosis in returning travelers has not yet been described in literature. The study group included 16 male and 5 female patients who traveled to Asian or Latin American countries for varying periods of time (Average: 4.04 months). In all patients, the first boil appeared towards the end of the trip and continued for several months after returning home. The average duration of disease (from first to last boil) lasted 8.38 months (Range 2-16 months) with an average of 4.23 recurrences (Range: 3-7). As the disease course progressed, subsequent recurrences became shorter and milder with longer interrecurrence intervals. Out of the 21 patients, only one patient described similar symptoms in companion travelers. Contributing factors, prior to the appearance of the first boil, were reported by 11 patients (52%) and included not showering for several days, sleeping in close proximity to a water source and insect bites. MSSA was isolated in 76.5% of patients, MRSA in 11.7% and Citobacter in 0.6%. In 17.6% of the patients, cultures were negative. Nasal colonization was demonstrated in 8 out 17 patients (47%). We discuss the possible infectious causes – virulent strain, nasal carriage or the possibility of an immune changing event secondary to exogenous factors contributing to an individual's decrease in immune protection.

Thailand- Not just a pretty place – Clinical case and review of the literature

Michal Katzir, MD and Michal Chowers, MD

Infectious disease unit Meir Medical center, Israel

International travelling has challenged us to diagnose and treat unfamiliar infections.
We present here a 24 years old man with complains of fever and right scapular pain radiating to his hand. Past history is positive for IDDM known for three years, very poor compliance with treatment.
He presented to us 10 days after returning from a six-week stay in south Thailand.
In Thailand after stopping his insulin therapy he was hospitalized in a local hospital due to the scapular pain and vomiting, discharge diagnosis was diabetic keto-acidosis and dehydration. No workup was done.
On admission, the patient was febrile. On chest X ray, two cavitary lesions were seen.



A diagnostic procedure was done.

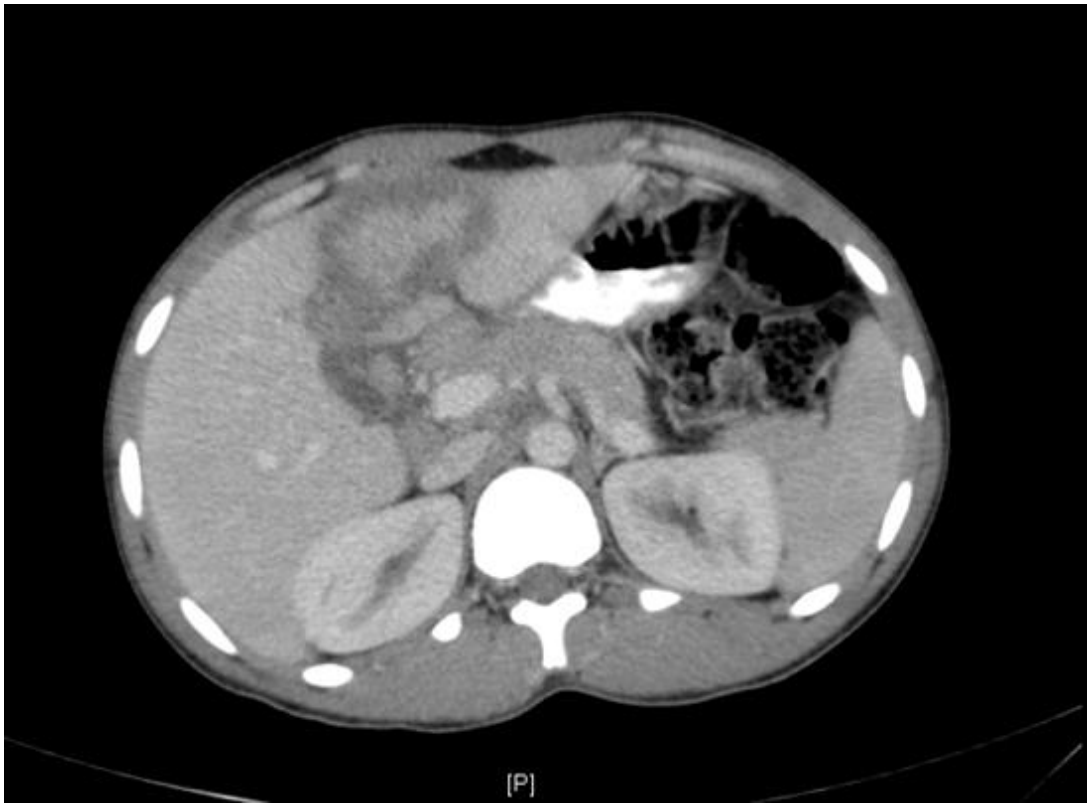
Not always what it looks like – A refugee from Eritrea with a tumor-like mass in the liver

Yael Paran & Steve Berger

Infectious Diseases Unit, Sourasky Medical Center, Tel Aviv

A 22-year-old male refugee from Eritrea who came to Israel 1.5 months prior to admission, presented to the emergency room with a two week history of right upper abdominal pain and a temperature of 37.5° C. On physical examination, he was in good general condition. There were tenderness and guarding in the right upper quadrant of his abdomen. His laboratory tests showed mild anemia with a hemoglobin concentration of 11.3 g/dl, a leukocyte count of 8300 / μL with marked peripheral eosinophilia of 33% (total 2800 / μL) and a platelet count of 499,000 / uL. There was a mild elevation of cholestatic liver enzymes; and the AST, ALT and bilirubin levels were within normal limits. An abdominal CT scan revealed a space-occupying lesion 5 cm X 7 cm X 4 cm in the left lobe of the liver. The lesion showed heterogeneous density, with central enhancement in the portal phase. There was also a finding suspicious of portal vein thrombosis (Fig. 1). The space-occupying lesion was suspected to be a primary neoplasm of the liver. Due to signs of central hemorrhage in the mass with a high risk of bleeding, a decision was made to proceed investigation with an abdominal laparoscopy. During laparoscopy, multiple small lesions were seen scattered on the liver. The mass was removed.

What is the diagnosis?



The changing epidemiology of human African tryponasomiasis among patients from non-endemic countries: 1902-2012

Neuberger Ami[1], Meltzer Eyal[2], Leshem Eyal[2], Dickstein Yaakov[3], Steinlauf Shmuel[2], Schwartz Eli[2]

[1]Internal Medicine B and the Unit of Infectious Diseases, Rambam Medical Center, Haifa, Israel; [2]The Center for Geographic Medicine and Department of Medicine C, The Chaim Sheba Medical Center, Tel Hashomer, Israel; [3]Internal Medicine A, Rambam Medical Center, Haifa, Israel

Background: Human African trypanosomiasis (HAT) is caused by the protozoan parasite *Trypanosoma brucei*, and transmitted by the bite of the tsetse fly. Although considered rare among travelers, there has been an increase in the number of reported HAT cases among patients from non-endemic countries (NEC). In light of the political, economic, and epidemiologic changes that occurred in Africa since colonial times, we have sought to describe the epidemiology of HAT in patients from NEC during the past 110 years.
Methods: Data for this article were identified by searching the public databases. All articles written in English, French and German were included. Only HAT cases involving patients from NEC were included. Data regarding the number of visitors to endemic countries were obtained from the United Nations World Tourism Organization (UNWTO).
Results: Between the years 1902 and 2012 224 cases of HAT were reported in patients from NEC. Relatively more cases of HAT were reported in the first two decades of the 20th century (an average of /decade) and the period between 1991 through 2010 (an average of 46/decade). Only 19 cases were reported between the years 1921 and 1965. When the "colonial" and "post-colonial" periods were compared, the average age of patients diagnosed before 1967 was lower than the age of patients diagnosed afterwards (32.5±7.8 and 43.0±16.1, respectively, $p<0.001$). In addition, between 1902 and 1966 11/109 (10.1%) of patients were female, and this proportion increased to 30/130 (23.1%) after 1966 ($p<0.001$). Until the 1960s most cases of HAT were diagnosed in expatriates (34/86, 39.5%), missionaries (15/86, 17.4%), and soldiers (15/86, 17.4%). However, after 1966 the vast majority of patients were tourists (91/125, 72.8%). During the "post-colonial" period fewer cases have been diagnosed in West Africa, and instead most cases are diagnosed among tourists visiting game parks in East or Central Africa, most commonly in Tanzania. *Trypanosoma brucei rhodesiense* accounted for 94/122 (77%) of all cases diagnosed after 1966, whereas most cases of HAT reported during the first half of the 20th century were acquired in countries where only *Trypanosoma brucei gambiense* is endemic. In most countries where cases of HAT among tourists have been reported between 1995 and 2010, a linear increase in the number of visitors from NEC was reported by the UNWTO. The change in the number of reported cases per year, however, does not correspond linearly to this increase. Rather, single cases continue to occur sporadically, interspaced with small outbreaks.
Conclusions: The epidemiology of HAT among patients from NEC has changed. Patients in recent decades are more likely to be short-term tourists, are older, more likely

to be female, and more likely to have acquired the disease while visiting game-parks in East Africa. Rhodesiense trypanosomiasis now accounts for the vast majority of HAT cases, whereas the opposite was correct at the turn of the 20th century. The tendency of East African trypanosomiasis to occur in relatively small "pockets" or "hot spots" of endemicity is related to HAT outbreaks among tourists visiting the game parks of East Africa. Any febrile patient with a compatible travel history and without an alternative diagnosis should be evaluated for HAT.

"Tropical" and unusual infections among undocumented migrants who are treated at the designated clinics in Tel-Aviv, Israel

Zohar Mor[1], Nadav Davidovitch[2], Ido Lurie[3], Alex Leventhal[4,5], Michael Dor[6], Itamar Grotto[2,7]

1- Ramla Health Department, Ministry of Health; 2- Faculty of Medicine, Ben Gurion University in the Negev, Beer Sheva; 3- Physician for Human Rights, Tel Aviv; 4- Department of International Relations, Ministry of Health, Jerusalem; 5- School of Public Health, Hebrew University-Hadassah, Jerusalem; 6- Medical Administration, Jerusalem; 7- Public Health Services, Ministry of Health

Background: The burden of "tropical" morbidity among migrants originating in the horn of Africa and reside in Israel in yet unknown. However, it is estimated that they reflect similar disease burden as reported from their countries of origin, which is higher than known in Israel. Public officials in Israel have expressed their concern with regards to the possibility of disease transmission from the migrants to the hosting population and also to the cost of medical treatment.

The aim of this study is to assess the incidence rate of "tropical" infections among migrants originating in the horn of Africa who are treated at the designated community clinics operating in Tel-Aviv, Israel. The clinics are operated by Physicians for Human Rights and by the Ministry of Health- Israeli Medical Association- Magen David Adom, and provide free basic medical services to migrants who are excluded from the National Medical Insurance Law, as they are not citizens.

Methods: A random sample of all medical files from the two community clinics for migrants treated between 2008 and 2010. Demographic details, major complaints, diagnosis and treatment regimens were collected for each of the files sampled.

Results: During the study period, ~11,000 migrants who visited the 2 designated clinics, while 6422 (58.4%) originated in horn of Africa. Of those, 309 were randomly sampled. Most of the migrants were males (N=239, 78.6%), the average age was 29 years and in average 11 months have elapsed from their arrival in Israel to their first clinic visit.

"Tropical" and other unusual infections in adults were detected as followed: one (0.3%) had schistosomiasis, one (0.3%) had brain toxoplasmosis, two (0.6%) had varicella zoster, two (0.6%) had herpes zoster, two (0.6%) had malaria, and five (1.6%) cases of intestinal worms. The later were treated with vermox until cure. The migrants who had brain toxoplasmosis and varicella zoster were co-infected with HIV who were neither followed-up nor treated in the AIDS centers due to lack of accessibility.

Conclusions: This study provides data with regards to the burden of "tropical" infection in a cohort of migrants from the horn of Africa who are treated in the designated clinics in Tel-Aviv. It is estimated that the burden among those migrants is higher than that of Israeli citizens. Although "tropical" morbidity in Israel is rare, a simple therapy provides cure, and therefore high index of suspicion and early diagnosis is crucial in protecting public health. We therefore stress the importance of ensuring free access to medical services. Some of the infections were detected in HIV-infected migrant and it is probable that routing follow-up and treatment in the AIDS centers would have prevented co-infections. Although it is difficult to compare morbidity rate between migrants and Israeli citizens due to lack of routine surveillance, this study provide a valid estimation of morbidity among migrants from the horn of Africa.

14:00 – 16:00 **Session 4: General Parasitology**

Chairs: Joseph El-On & Gad Baneth

14:00 – 14:15 **Increasing in rate of detection of enteric parasites by using different diagnostic methods** - Elsa R. Pavlotzky, Lilia Milner, Svetlana Shpraga & Shifra Ken-Dror

14:15 – 14:30 **Immunomodulatory effect of the tellurium compound AS101 on *Leishmania major* promastigotes and amastigotes in culture and in experimentally infected mice** - P. Berenstein, B. Sredni & J. El-On

14:45 – 15:00 **Detection and molecular identification of *Ixodes ricinus* on beef cattle in Israel** - Oran Erster, Asael Roth, Yuval Hadani & Varda Shkap

15:00 – 15:15 **Mitochondrial markers as a tool for tick identification and phylogenetic analysis** - Oran Erster, Asael Roth, Ricardo Wollkomirsky, Benny Leibovich & Varda Shkap

15:15 – 15:30 **Molecular identification of *Rickettsia aeschlimannii* and *Rickettsia africae* in *Hyalomma* spp. ticks and camels from Israel** - Gabriela Kleinerman, Gad Baneth, Kosta Y. Mumcuoglu, Michael van Straten, Dalia Berlin, Dmitry A. Apanaskevich & Shimon Harrus

15:30 – 15:45 **Prevalence of *Bartonella* species in stray and domestic cats from Israel** - Ricardo Gutierrez, Danny Morick, Ifat Gross, Ronen Winkler & Shimon Harrus

15:45 – 16:00 **Discussion**

Increasing in rate of detection of enteric parasites by using different diagnostic methods

Elsa R. Pavlotzky, Lilia Milner, Svetlana Shpraga and Shifra Ken-Dror

Parasitology Unit, Microbiology Laboratory, Haifa and Western Galilee Laboratory, Clalit Health Services

Routine diagnostic parasitology generally includes laboratory procedures for detection of organisms in clinical specimens by using morphological criteria. The most commonly performed procedure in parasitology is the ova and parasite examination which is composed of three separate protocols: direct wet mount, concentration and permanent stained smear. In practice, it is not always possible to perform all the three methods due to the shortage of man power for processing the huge number of samples. In our laboratory, it was decided to change the laborious and time consuming method of permanent stained smears to the stool culture method in case of soft or semiformed specimens, and perform concentration method in formed samples. Culture methods in appropriate medium and with suitable quality control can help detect trophozoites protozoa, amebae and flagellates.

In the present study, human stool samples collected from the Haifa and Western Galilee area during January and February, 2012 were examined. Out of 1,687 stool samples, 570 were tested by culture method and 1,117 by concentration method, while all samples were examined by direct wet mount as well. It was shown that the yield of intestinal protozoa such as *Blastocystis spp* and *Dientamoeba fragilis* increased by 30% and 19%, respectively with an additional stool culture examination. The concentration method elevated the detection of *Entamoeba coli* and *Giardia lamblia* by 25% and 23%, respectively. The culture method added 48% to positive samples and the concentration method added 36% in comparison to direct wet method alone. The most frequent parasites were *Blastocystis* spp. and *Dientamoeba fragilis* (54.4% and 21.1%. respectively). The present data show that the use of several methods jointly increases the rate of detection of enteric parasites.

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Immunomodulatory effect of the tellurium compound AS101 on *Leishmania major* promastigotes and amastigotes in culture and in experimentally infected mice

P. Berenstein[1], B. Sredni[1], J. El-On[2]

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In leishmaniasis, the immunological state of the host appears to play an important role in the clinical pattern of the disease and on the efficacy of treatment. The aims of this study was to determine the efficacy of AS101 [ammonium tri-chloro(dioxoethylene-O,O'-) tellurate], a novel immune-response-activating agent, either alone or combined with either Pentostam® (PEN) or paromomycin PR) on *Leishmania major* development *in vitro* and *in vivo* in experimentally infected mice. AS101 was highly effective against *Leishmania* promastigotes (IC_{50}=5µg/ml) and amastigotes (IC_{50}=0.1 µg/ml). On the 3rd day of treatment, AS101 at 0.5, 5, and 10µg/ml inhibited the amatigotes development by 65.9%, 80.2%, and 99.4%, respectively. However, total elimination of the intracellular parasites was not achieved with AS101with neither of the concentrations used. Pre-treatment of *L. major* promastigotes with AS101 (5, 10µg/ml) reduced their infectivity (52.6%, 72.8%) to the macrophage. Combination chemotherapy of AS101 and either PEN or PR showed a synergistic effect. AS-101 was shown to switch the immune response of infected cells to T-helper 1-oriented by inhibiting IL-10 and increasing IL-12 production. The possible mechanism of IL-12 induction by AS101 is supposed to be via the direct inhibition of pAkt protein, causing mTOR down-regulation. In this work we have shown for the first time the ability of AS101 to inhibit TOR1, essential for parasites' metabolism. AS101 was found to induce an apoptotic effect, demonstrated by changes in flagella pocket and the appearance of multi-lamellar bodies, bi-layered nuclei membranes, apoptotic lipid vesicles and nucleus destruction, as determined by a transmission electron microscopy. A decrease in parasites load and a delay in mice mortality was observed in infected Balb/c mice treated with AS101 combined with either Leshcutan (15% PR + 12% MBCL in soft white paraffin) or PR.The present study suggests the use of AS101 as an adjunct to existing anti-leishmanial drugs in the treatment of the disease.

Detection and molecular identification of *Ixodes ricinus* on beef cattle in Israel

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The three-host tick *Ixodes ricinus* (Acari: Ixodidae), also known as the castor bean tick, is an important vector of several veterinary and zoonotic diseases. The geographic distribution of *I. ricinus* extends from Scandinavia in the north of Europe to the Atlas Mountains in Morocco in the south. Up to now, in the Middle East this tick was considered to be confined to Turkey and northern Iran. Here, we report for the first time on the presence of *I. ricinus* on beef cattle in Israel. Tick samples were collected from field-grazing beef cattle in western Galilee (northern Israel) during the winter months of 2011-2012. The samples were first examined morphologically for species-specific taxonomical features and then by molecular characterization. Ticks identified morphologically as *I. ricinus* were then examined by PCR with four different molecular markers: 12S rRNA, 16S rRNA, COX1 and cytochrome B. The PCR products were sequenced and compared with annotated *I. ricinus* sequences in GenBank™ and the analyzed sequences from the collected samples shared 98–99% identity with reported *I. ricinus* sequences. In contrast, sequences from the collected ticks shared identity of 91% or less with annotated sequences from other *Ixodes* species. Multiple alignments and neighbor-joining analyses performed for each of the four markers reinforced the results obtained from pairwise alignments. These findings demonstrated for the first time the presence in Israel of the tick species *I. ricinus* – with results confirmed by a combination of morphological examination and molecular analyses.

Mitochondrial markers as a tool for tick identification and phylogenetic analysis

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The sequences of four mitochondrial gene segments of four *Rhipicephalus* tick species were compared: *R. annulatus*, *R. bursa*, *R. sanguineus* and *R. turanicus*. These are economically-important ticks that parasitize domestic animals and transmit veterinary and zoonotic diseases. Gene segments from the genes encoding 12S rRNA, 16S rRNA, cytochrome C oxidase subunit 1 (COX1), and cytochrome B (CytB) were amplified using consensus primers and analyzed for unique restriction sites. Where unique digestion patterns were found, species-specific digestions were performed, so that each tick species could be identified by its unique restriction pattern. The marker sequences were also used for a preliminary phylogeny study, in which orthologous sequences of each marker were compared and genetic distances between each species were deduced. For the 12S rRNA marker, unique digestions were identified for the *R. annulatus* and *R. bursa* sequences. For the COX1 marker, specific sites were identified for *R. annulatus*, *R. bursa* and *R. sanguineus* sequences. For the CytB marker, unique digestion patterns were found for each of the four markers. No species-specific patterns were found for the 16S marker. An ongoing work is now being performed on samples from multiple *Hyalomma* species, using the same markers, to detect species-specific restriction patterns that would enable quick identification of *Hyalomma* field samples. Our results demonstrate that related tick species could be distinguished by amplification of selected genetic markers using consensus primers, followed by species-specific digestions. With sufficient sequence information, a combination of multiple digestions, i.e. several markers for the same species sample, can determine the species of the sample without the need for morphological characterization and without the need to sequence the PCR products. The data also suggest that these marker sequences could be used for a preliminary study of the genetic relationship between these species.

Molecular identification of *Rickettsia aeschlimannii* and *Rickettsia africae* in *Hyalomma* spp. ticks and camels from Israel

Gabriela Kleinerman[1], Gad Baneth[1], Kosta Y. Mumcuoglu[2], Michael van Straten[1], Dalia Berlin[1], Dmitry A. Apanaskevich[3] and Shimon Harrus[1]

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Background: Israel is an endemic region for Spotted Fever Group (SFG) rickettsiae, and clinical cases are commonly reported in this country. The main agent of SFG rickettsiosis in Israel is *Rickettsia conorii israelensis*, however several other species of SFG rickettsiae including *Rickettsia masilliae* and *Rickettsia sibirica mongolitimonae* have been recently detected in questing ticks. *Rickettsia aeschlimannii* was also detected in *Hyalomma marginatum* and *Hyalomma scupense* (= *Hyalomma detritum*) ticks collected from wild animals in Israel. In this study we aimed to identify and genetically characterize SFG rickettsiae in ticks and domestic animals, mainly camels from farms and Bedouin communities in the Negev desert.
Methods: The study included collection of ticks and blood samples from 5 different locations in the Negev and the Dead Sea area during the years 2007 and 2012. DNA was extracted from the samples and molecular diagnosis was performed. Initial screening of rickettsiae was carried out by targeting the *gltA* gene. In order to discriminate between the different SFG *Rickettsia* spp., positive samples were further analyzed for the presence of the *ompA, 17kDA*, *ompB* and *16S* rickettsial gene fragments.
Results: Four hundred and forty nine ixodid ticks collected from camels, horses, and a dog, as well as 152 blood samples from camels (n:148) and horses (n:4) were included in this study. A 200bp rickettsial *gltA* gene fragment was amplified in 23 of a total of 449 samples, in all localities except for Arad. Amplified sequences identical to *Rickettsia aeschlimannii* were detected targeting the *ompA, 17kDA* and *ompB* genes in gltA positive DNA samples. The amplicons had an identity of 94-99%, and were found in *Hyalomma dromedarii, Hyalomma turanicum, Hyalomma excavatum* and *Hyalomma impeltatum* ticks collected from camels from the Dead Sea, Dimona and Avdat regions. Two amplified fragments of 630 base pairs similar to *Rickettsia africae* were obtained when targeting the *ompA* gene with 99% sequence identity. The latter was found in *H. dromedarii* and *H. impeltatum* ticks parasitizing a horse in the Dead Sea and a camel from a farm in Dimona, respectively. One *ompB* gene fragment from a *H. turanicum*, revealed an amplicon of 845 bp. which had 98% sequence similarity to *R. sibirica mongolitimonae*. Three *ompA* gene sequences similar to *R. aeschlimannii* were detected in three camels with 99-100% identity.
Conclusions: This is the first report documenting the presence of *R. aeschlimannii* and *R. africae* in *Hyalomma* spp. ticks collected from camels from Israel, and the first report of *R. africae* in Israel. The findings presented in this study have public health implications. Physicians should take these findings into consideration when Bedouin and other patients

are presented with fever or other clinical signs compatible with rickettsioses or with signs of fever of unknown origin, considering that some populations are in continuous exposure to ticks parasitizing their domestic animals and consequently in risk of contracting rickettsial infections.

Prevalence of *Bartonella* species in stray and domestic cats from Israel

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Background: Cats act as ideal reservoirs for *Bartonella* species, maintaining often-asymptomatic bacteremia for prolonged periods (months). The most common *Bartonella* species detected in cats are *B. henselae* and *B. clarridgeiae* and both have been implicated as the cause of cat-scratch disease (CSD) and bacillary angiomatosis in humans. A third specie, *B. koehlerae*, has also been isolated from cats and implicated in a human case of endocarditis from Israel, but its distribution within Israeli feline populations still remains to be determined. The prevalence of *Bartonella* spp. in cats varies between geographic locations and cat populations. The present study investigated the prevalence of *Bartonella* spp. in blood of domestic and stray cats, from the central and north-central Israel, and compares molecular methods with culture isolation in the diagnosis of bartonellosis.
Methods: Three hundred and thirty four EDTA blood samples were collected from 179 stray cats and 155 domestic cats from 18 urban areas and their surroundings in Israel. All samples were screened for *Bartonella* spp. infection by two different detection methods: culture-isolation of *Bartonella* spp. in chocolate agar and molecular detection of *Bartonella*-DNA using a high resolution melt (HRM) real-time PCR assay that targeted the 16S-23S intergenic spacer (ITS). All positive samples were confirmed by two other real-time PCR assays targeting the *rpoB* and *16S* gene fragments.
Results: *Bartonella* was detected in 30.7% (55/179) of the stray cats and 18.7% (29/155) of the domestic cats, by one or both detection methods. The prevalence of *Bartonella* spp. was significantly higher in stray cats than in domestic cats ($P = 0.012$). Overall, the species identified in both cat populations were *B. henselae*, *B. clarridgeiae* and *B. koehlerae* [15.6% (52/334), 8.1% (37/334) and 3.6% (12/334), respectively]. No significant difference was noticed in the prevalence of *B. henselae* between stray and domestic cats [15.6% (28/179) and 15.5% (24/155), respectively ($P = 0.968$)]. However, in stray cats, *B. clarridgeiae* and *B. koehlerae* had a significantly higher prevalence, 12.3% (22/179) and 5.6% (10/179), respectively, compared to a 3.2% (5/155) and 1.2% (2/155), in domestic cats, respectively ($P = 0.002$ and 0.036, respectively). Co-infection was determined in 2.1% (7 cats): *B. henselae* and *B. clarridgeiae* (3 cats), *B. henselae* and *B. koehlerae* (3 cats), and *B. clarridgeiae* and *B. koehlerae* (1 cat). Five of these co-infection cases were detected in stray cats and 2 in domestic cats.
Discussion and Conclusions: This study demonstrates the high prevalence of *B. henselae*, *B. clarridgeiae* and *B. koehlerae* within stray and domestic cats from Israel. The ITS real-time PCR showed a high screening power, detecting the 94.0% of all positive samples (79/84). In contrast, bacterial isolation was not shown to be sensitive in detecting *Bartonella* positive feline blood samples (35.7%, 30/84). The high prevalence of these *Bartonella* species, coupled with their zoonotic potential and the overpopulation of stray cats in major urban centers such as Tel Aviv and Jerusalem, represent a significant threat for public health in this country.