ABSTRACTS

Thursday, December 15, 2011

09:00 -10:00 **Plenary lectures I: From the future science back to Mother Nature**

09:00 -09:30 ***Daniel Zilberstein*** **– Back to the future: a journey from classical parasitology to molecular biology and back**

09:30 -10:00 ***Kosta Y. Mumcuoglu*** **– Biotherapy: The use of maggots and leeches in the treatment of chronic and acute wounds**

Back to the future: a journey from classical parasitology to molecular biology and back

Dan Zilberstein

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The revolution of genetic engineering in the 1970s and high throughput technologies in the 1990s had an enormous effect on the development of the field of parasitology. Up to the late 1970s research in our filed focused mostly on parasite life cycles, basic biochemistry and host immune response. Gene cloning in the 1980s revived the field by enabling insight into parasite cell biology and molecules involved in host parasite interactions. High throughput techniques led to the publication of parasite genomes that was followed by massive transcriptomics and proteomics analyses. However, albeit the great contribution of these technologies, we still do not know much about the cell cycle of most parasitic protozoa, vaccines are not available and we lack nontoxic drugs. The aim of my talk is to update on, and summarize on what has been achieved using molecular biology as well as to discuss future opportunities for our field.

Maggot therapy for the treatment of chronic wounds

Kosta Y. Mumcuoglu

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Maggot debridement therapy (MDT) is the intentional treatment of suppurative skin infections with the larvae of the green bottle fly, *Lucilia sericata.* Today, this treatment modality is being used in over 30 countries worldwide and in the last 20 years, more than 80,000 patients have been treated in 2,000 medical institutions worldwide. Since 1996, 550 patients with 880 wounds were treated in Israel, most of them in the Hadassah Medical Center, Jerusalem. Half of the patients were treated ambulatory, and the other half while hospitalized. Half of the patients had diabetic food ulcers, while an additional 25% had ulcers due to vascular diseases. Approximately 1/3 of the patients with leg ulceration were potential candidates for amputation or major surgical interventions. The number of treatments varied between 1-8 (median: 2, average: 3.6) and the number of treatment days between 1-14 days (median: 3, average: 6.3). In 80% of the cases, the wound was debrided completely while in additional 15% a partial debridement was achieved. In at least 55 cases an eminent amputation was prevented due to positive results of MDT. In other cases, a more proximal amputation could be avoided. Some scientific aspects of the mechanisms involved in the debridement of the wound, as well as of the antibacterials secreted/excreted by the maggots will be discussed.

Hirudotherapy (HT) is the used of leeches (*Hirudo medicinalis*) for the treatment of acute wounds. Since 2003, in collaboration with physicians in the Department of Plastic Surgery of the Hadassah University Hospital, Jerusalem, 33 patients, 8-79 years old presenting with venous congestion of revascularized or replanted fingers, free or local flaps were treated by leech therapy. Of the 25 fingers, 17 fingers were saved, while 22 out of 23 flaps were salvaged. The use of the medicinal leech can be complicated by infections caused by Aeromonas spp., which are leech endosymbionts. Accordingly, patients undergoing hirudotherapy receive systemic chemoprophylaxis. In order to render leeches safe for use on patients, leeches were fed artificially with ciprofloxacin using an arginine solution as a phagostimulant. Aeromonads were detected in 57 of 80 control leeches (71.3%), but in none of the 56 leeches treated with ciprofloxacin ($p<0.001$). Treated leeches survived for up to 4 months. Tested weekly, 61% of these leeches took human blood for at least 4 weeks after treatment and all remained negative for aeromonads. All water samples in which leeches were kept before treatment were contaminated with Aeromonas spp., while none was detected in any of the NaCl/arginine solutions on which treated animals were fed. Molecular characterization of two phenotypically different isolates by gyrB sequencing showed that one clustered tightly with *Aeromonas veronii* and the other was closely related to *Aeromonas media*. Ciprofloxacin reduced the number of leech-associated aeromonads to undetectable levels for extended periods. Most treated leeches were ready to take a blood meal after treatment, suggesting the possibility of using ciprofloxacin treated leeches instead of chemoprophylaxis in HT.

10:35 - 11:35 **Plenary lectures II. Global world and globalization of diseases**

10:35 - 11:05 ***Robert Steffen (Switzerland)*** **- Infectious diseases - A continued challenge in a globalized world**

11:05 - 11:35 ***Peter Rabinowitz (USA)*** **- One health and infections: Evidence for integrating human, animal and environmental aspects**

Infectious diseases — A continued challenge in a globalized world

Robert Steffen

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Impressed by the success of antibiotic therapy and smallpox eradication, the Surgeon General of the United States declared in 1967 “The time has come to close the book on infectious diseases“ — an illusion as demonstrated by the subsequent HIV pandemic and in this new century by epidemics or pandemics associated with Severe Acute Respiratory Syndrome (SARS), Influenza H1N1v2009, and in the past summer by entero (aggregative) hemorrhagic E. coli (EHEC or EAHEC). Also cholera, dengue, chikungunya and measles outbreaks were reported in some countries. How to explain these emerging and re-emerging infections? What to expect after a look into the crystal ball? The globe always had to face infectious threats as pathogens have more genetic options to adapt to a changing environment — no chance for humans to win in that race. In view of urbanization, increasing intercontinental travel potentially resulting in rapid global spread, excessive misuse of antibiotics resulting in resistance, and lack of compliance with immunizations an increasing number of events that may constitute a public health emergency of international concern should not come as a surprise. Also improved diagnostic means and far better surveillance options basing on the revised International Health Regulations may have resulted in an increased number of outbreak reports. Host factors such as malnutrition in some parts of the world, or also the increasing therapeutic use of immunosuppressive agents or immunosenescence in industrialized countries with a growing proportion of older adults may also play a role. Lastly global climate change — besides non-infectious impacts such as destruction of crops and infrastructure — will continue to result in specific infectious threats and in a few areas in benefits. In

contrast to common opinion one to two decades ago, large-scale bioterrorist attacks are considered less likely. In conclusion, those in charge of surveillance and response of communicable diseases will not get bored.

One health and infections: evidence for integrating human, animal, and environmental aspects

Peter Rabinowitz

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Recently there has been widespread discussion of a "One Health" approach to infectious diseases that are occurring at the intersection of human, animal, and environmental health. This approach recognizes the interconnectedness of environmental change and disease emergence in animal and human populations, and the need for an intersectoral effort to prevent and control these diseases. A number of international declarations and conferences, such as the tripartite declaration of the World Health Organization (WHO), the UN Food and Agriculture Organization (FAO), and the Organisation for Animal Health (OIE), the Stone Mountain Conference on One Health, and the First International One Health Congress have proposed future directions for such efforts. While interpretations differ about the exact meaning of the One Health concept, most agree that it highlights the importance of considering concurrently the human, animal, and environmental aspects of an infectious disease issue. But does this concept differ from previous approaches, and if so, what is the evidence that it adds value? A critical review of the biomedical literature on disease interventions reveal surprisingly few that track the impact of interventions on human, animal, and environmental outcomes, but also suggest the critical value of such an integrated approach.

12:00 - 13:35 **Session I: Travel Medicine**

12:00 – 12:30 ***Robert Steffen (Switzerland)*** **- Neglected neurological Infections in international travelers: Meningococcal disease and Japanese encephalitis**

12:55 – 13:10 ***Eyal Leshem, Eyal Meltzer, Shmuel Stienlauf, Eran Kopel and Eli Schwartz*** **- Efficacy of short prophylactic course of atovaquone- proguanil (Malarone®)**

13:10 – 13:25 ***Rami N. Sammour, Rabia Bahous, Moti Grupper, Gonen Ohel, Shmuel Steinlauf, Eli Schwartz and Israel Potasman*** **- Pregnancy course and outcome in women traveling to developing countries**

13:25 – 13:35 ***Jacob Moran-Gilad Emilia Anis and Daniele Goldmann*** **- Ministry of Health guidelines for international travel – update 2011**

Neglected neurological infections in international travelers: Meningococcal disease and Japanese encephalitis

Robert Steffen

Division of Epidemiology and Prevention of Communicable Diseases, WHO Collaborating Centre for Travellers' Health, University Institute of Social and Preventive Medicine, Zurich, Switzerland and Division of Epidemiology, Human Genetics & Environmental Sciences, University of Texas School of Public Health, Houston, TX, U.S.A.

Travel-related neurological syndromes are rarely diagnosed; according to Geosentinel surveys 1% of the patients consult for neurological disorders, most of them non-infectious. According to a French survey, among 42 patients with confirmed etiology of meningo-encephalitis, 24 were of viral (11 due to Enteroviruses, 8 to Herpesviridae) and 4 of bacterial origin, while 12 were associated with malaria and 2 with other parasites. Few infectious diseases mainly causing neurological symptoms are vaccine preventable: Rabies, poliomyelitis, meningococcal disease, and Japanese encephalitis (JE). As new vaccines have now been introduced against the latter two, we will concentrate on those. JE is the leading cause of viral neurological infections in Asia, some 50,000 symptomatic cases are annually reported from endemic countries. The case fatality rate is about

30%, another third of the patients is left with sequelae, while only the remaining third has a full recovery. In the 1973 to 2008 period, 55 travelers with JE have been recorded. On this basis a rate of 1 per 300.000 Scandinavian travelers or 1 per million travelers of any nationality with destinations in endemic countries have been estimated, but we must take underreporting into account. Most patients were tourists, several with brief beach vacations, a few were infected outside the season of transmission. Recently a new vaccine containing inactivated Japanese encephalitis virus (purified JEV proteins from attenuated strain SA_{14}-14-2) produced in Vero cells and adsorbed on aluminum hydroxide has been introcuced (IXIARO®, JESPECT®, formerly IC51). It has been demonstrated to be efficient and has a far better toxicity profile as compared to vaccines previously used in industrialized countries. An algorithm for its use in travelers will be proposed. Meningococcal disease similarly to JE is rare in travelers and overall is recorded in about 1 per million. It also may have a severe impact resulting in tragedies. Although a few risk factors are known, it now more often affects travelers without obvious risks. New conjugate vaccines effective against serogroups A, C, W135 and Y confer multiple advantages as compared to the older polysaccharide vaccines or mono- / bi-valent brands. Particularly they are effective in infants and there is no hyporesponsiveness with repeated dosing. Again, an algorithm for its use in travelers will be proposed, taking into consideration those at highest risk. In conclusion, it is inadequate to set travel vaccines priorities only on the base of incidence rates, the impact of infection may be just as important. As in both infections, defined risk groups play a minor role, cumulative risk exposure becomes more important.

Efficacy of short prophylactic course of atovaquone- proguanil (Malarone®)

Eyal Leshem, Eyal Meltzer, Shmuel Stienlauf, Eran Kopel and Eli Schwartz

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Background: Atovaquone combined with proguanil hydrochloride (AP, Malarone) is a relatively new falciparum malaria prophylactic drug, active during the liver stage of the parasite (causal prophylaxis). This activity against hepatic schizonts obviates the need to continue therapy for one month after exposure ends (as required in blood stage prophylactic agents). Current guidelines recommend continuation of AP prophylaxis for seven days after leaving endemic areas. Evidence from previous studies suggest that continuation for one day after malaria exposure ends may be sufficient. We conducted a retrospective survey of travelers to malaria endemic areas who used this short prophylaxis course to identify falciparum malaria cases among these travelers.

Methods: *Active surveillance*: A retrospective survey of travelers to malaria highly endemic areas (sub-Saharan Africa only). Travelers, who visited the pre-travel clinic at the Sheba Medical center and were recommended to use AP prophylaxis, were included. The travelers were contacted and questioned regarding prophylaxis adherence, duration, and malaria diagnosis during or after travel. *Passive surveillance*: All cases of malaria are reported to the Israel Ministry of Health (MOH) malaria registry by law. A retrospective analysis was performed looking at all falciparum malaria cases reported to the MOH since 2003 (year in which AP was licensed in Israel). Information about chemoprophylaxis use among these patients, especially of using short course of AP, was retrieved.

Results: The telephone survey study included 134 travelers to endemic countries between the years 2010-2011. The study population traveled a total of 1520 days in endemic areas. Among 134 travelers, 90% discontinued AP one day after leaving the malaria endemic areas. No cases of malaria were noted in this population.
The MOH registry survey included 118 falciparum patients between the years 2003-2008. The majority (100; 85%) did not take any malaria prophylactic. None of the patients had used malaria prophylaxis with AP (neither regular nor short AP course.)
Conclusions: We retrospectively studied a large group of travelers exposed to highly endemic malaria areas. Despite cessation of AP prophylaxis one day after leaving the endemic areas, none of the travelers developed malaria. In addition, analyzing the epidemiology data of malaria cases in Israel did not show any falciparum malaria case which occurred after AP prophylaxis (regular or short course). Based on pharmacokinetic properties and falciparum malaria pathophysiology it is reasonable to recommend use of AP prophylaxis ending one day after leaving the endemic area. Further prospective validation of our findings in larger number of travelers should follow.

Pregnancy course and outcome in women traveling to developing countries

Rami N. Sammour, M.D.[1], Rabia Bahous, M.D.[1], Moti Grupper, M.D.[2], Gonen Ohel, M.D.[1], Shmuel Steinlauf, M.D.[3], Eli Schwartz M.D.[3] and Israel Potasman, M.D.[2]

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Background: The issue of travel to developing countries during pregnancy has not been sufficiently studied, and such travel was discouraged by some authors, despite lack of evidence-based recommendations. The aim of this study is to investigate the rate, course and outcome of pregnancies in women who traveled to developing countries, or became pregnant during such travel.

Methods: Women visiting our two travel clinics for pre-travel consultation and vaccination during the years 2004-2009, and who declared a possibility or an intention of becoming pregnant during travel, were contacted. This was followed by a telephone interview by an obstetrician with those women who were actually pregnant. Background characteristics and data about pregnancy course, morbidity during travel, and pregnancy outcome were collected.

Results: A total of 52,430 medical records were screened for compatibility. Forty-six women met the inclusion criteria and consented to participate in the study, 33 of which were pregnant at departure and 13 conceived during travel. The incidence of pregnancy during travel was 0.93/1000 travelers. Thirty women traveled to Southeast Asia, 8 to South and Central America, 5 to Africa, 4 to Australia, and 1 to China. More then 2/3 of women received pre-travel vaccinations. Adherence to the WHO recommendations regarding food and drink was high (87%) and travelers' diarrhea occurred in only 11% of women. Five of 22 women traveling to malarious areas had taken antimalarial prophylaxis. Six women required medical therapy during travel. Pregnancy outcome was not different from the normal population except for an unusually low rate of preterm delivery.

Conclusions: To the best of our knowledge, this is the first report of pregnancy course and outcome in a cohort of pregnant women traveling to developing countries. In our subjects, travel to developing countries was not associated with adverse pregnancy outcome. Our study does not support previous recommendations to avoid travel to tropical countries during pregnancy. Larger studies are needed to support these findings.

Ministry of Health guidelines for international travel – update 2011
***Jacob Moran-Gilad*, Emilia Anis and Daniele Goldmann -No abstract available**

12:00 - 13:30 **Session 2: Molecular Parasitology**

12:00 – 12:15 ***Alexandra Zinoviev* and *Michal Shapira* - Two Ded1p homologs in Leishmania relate to translation initiation in a stage specific manner**

12:15 – 12:30 ***Daniel Yasur Landau*, *Charles L. Jaffe, Lior David and Gad Baneth* - *Leishmania infantum* isolates from Israeli dogs showing reduced susceptibility to Allopurinol treatment**

12:30 – 12:45 ***Noah Dahan-Pasternak*, *Michael Pe'er and Ron Dzikowski* - A nuclear pore component PfSec13 is found in the nucleus of Plasmodium falciparum both at the nuclear envelope and in the nucleoplasm where it seems to be associated with heterochromatin**

12:45 – 13:00 ***Yair Fastman* and *Ron Dzikowski* - Erasing the epigenetic memory and beginning to switch – the onset of antigenic switching of *var* genes in *Plasmodium falciparum***

13:00 – 13:15 ***Doreen Schlisselberg*, *Ehud Inbar and Dan Zilberstein* - The amino terminal domain of LdAAP24 determines transporter specificity in *Leishmania donovani* promastigotes**

13:15 – 13:30 ***Sophia Katz*, *Oded Kushnir, Ayala Tovy, Rama Siman Tov and Serge Ankri* - The *E. histolytica* methyl line binding protein EhMLBP serves as a bridge between environmental stress response and epigenetic regulation**

Two Ded1p homologs in *Leishmania* relate to translation initiation in a stage specific manner

Alexandra Zinoviev and Michal Shapira

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Leishmania are ancient eukaryote parasites that cause the different clinical manifestations of Leishmaniasis. Translation initiation machinery of these unicellular organisms undergoes changes upon transition from the fly vector into the mammalian host. The eIF4F, that includes LeishIF4E-4 and LeishIF4G-3, is functional in promastigotes. This complex is heat sensitive, and disassembles in amastigotes and the expression level of its key subunits is reduced, eliminating the cap-dependent translation or reducing it to a level that is undetectable. Unlike LeishIF4E-4, LeishIF4E-1 maintains its expression level and becomes active in this life form. However analysis of its binding partners suggests that it is part of a cap-independent pathway of translation, since it fails to interact with any of the six eIF4G homologs found in the *Leishmania* genome, which contain the typical MIF4G domain. In pull-down assays using tagged LeishIF4E-1 and LeishIF4E-4 we identified two DEAD-box RNA helicases. These show sequence homology and functionally complement the yeast protein, Ded1. Ded1p is known to be implicated translation initiation in numerous studies, although the mode of its function remains unclear. Ded1p is known to be essential in yeast, however silencing of neither LeishDed1p-1 nor LeishDed1p-2 caused growth defect in the procyclic form of *T. brucei*. Examination of protein expression pattern shows that LeishDed1p-2 is promastigote specific, while LeishDed1p-1 is amastigote specific. These results could suggest involvement of the two *Leishmania* Ded1p homologs in different modes of translation, cap-dependent for LeishDed1p-2 and cap-independent for LeishDed1p-1, with some overlap in function. These results show once more the divergence of the translational machinery of these ancient parasites.

***Leishmania infantum* isolates from Israeli dogs showing reduced susceptibility to Allopurinol treatment**

Yasur Landau, D.[1], Jaffe, C.L.[2], David, L.[3], Baneth, G.[1]

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Leishmania spp. are parasitic protozoans that infect mammals. There are 23 different *Leishmania* species, most of them zoonotic with animals serving as reservoirs for infection. The clinical syndromes caused by leishmaniasis in humans include cutaneous, mucocutaneous and visceral forms. The visceral form of the disease is potentially fatal, and is prevalent in Africa, Latin America, the Middle East and the Mediterranean basin. Dogs and wild canines are considered the main reservoir for human visceral *L. infantum* infection and they also suffer from a severe and fatal disease caused by this pathogen. A growing concern over the last decade has been the large number of drug resistant *Leishmania* isolates infecting humans and an effort is being made to uncover the genetic and biochemical basis of resistance mechanisms.

Very little information has been available on resistance to anti-leishmanial drugs in dogs. Allopurinol**,** alone or in combination with meglumine antimoniate, is the main drug used for treatment of canine leishmaniasis. Although it is helpful in improving the clinical signs of infection in treated animals, relapse of disease despite treatment is possible. In Israel, several dozens new cases of canine leishmaniasis are diagnosed each year. The majority of dogs detected are treated with allopurinol monotherapy. Preliminary results on the susceptibility *L. infantum* strains isolated from naturally infected dogs to allopurinol will be presented. *L. infantum* parasites were isolated and cultured from 3 groups of infected dogs: newly diagnosed untreated dogs; infected clinically healthy allopurinol treated dogs; and dogs that showed clinical relapse during treatment. Sensitivity to allopurinol was evaluated using an alamarBlue® (AbD Serotec, Oxford, UK) viability assay that measures parasite proliferation at different drug concentrations. The IC50 for each isolate was determined. Values for the treated relapsed group (3 isolates) were 3-fold higher than comparable values from the non-treated or clinically healthy groups (10 and 3 isolates respectively). This is the first laboratory-based report of possible resistance to allopurinol in dogs. These results substantiate the suspicion that allopurinol resistant parasites can develop in treated dogs, likely due to natural selection of resistant parasites in response to treatment by itself or to a combination of host and parasite factors. Furthermore, these results suggest that the reduced susceptibility to allopurinol may be linked to a clinical relapse. We next intend to enlarge our sample size, and see whether a correlation can be found between the allopurinol dosing regime, host factors and parasite IC50 concentrations. Results might pave the way towards better treatment of leishmaniasis in dogs as well as to understanding the defense mechanisms of this parasite towards effective measures against this major zoonotic infection.

Erasing the epigenetic memory and beginning to switch – the onset of antigenic switching of *var* genes in *Plasmodium falciparum*

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Antigenic variation in *Plasmodium falciparum* is regulated by transcriptional switches among members of the *var* gene family, each expressed in a mutually exclusive manner and encoding a different variant of the surface antigens collectively named PfEMP1. Antigenic switching begins when the first merozoites egress from the liver and reach the blood circulation. By erasing the epigenetic memory we created parasites with no *var* background, similar to merozoites that egress from the liver, in order to investigate the onset of antigenic switches at the early phase of infection. At the onset of switching, the *var* transcription pattern is heterogeneous with numerous genes transcribed at low levels including upsA *vars* that are rarely activated in growing cultures. *var2csa*, the gene implicated in pregnancy associated malaria, could not be completely silenced by promoter titration, suggesting an additional level of regulation. Analysis of subsequent *in vitro* switches shows that the probability of a gene to turn on or off is not associated with its chromosomal position or promoter type *per se* but on intrinsic properties of each gene. Finally, we show that fine tuned

reduction in *var* transcription increases their switch rate, indicating that transcriptional perturbation can alter antigenic switching.

The amino terminal domain of LdAAP24 determines transporter specificity in *Leishmania donovani* promastigotes

Doreen Schlisselberg, Ehud Inbar and Dan Zilberstein

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Parasitic protozoa of the genus *Leishmania* are the causative agents of a wide range of cutaneous, mucocutaneous and visceral diseases in humans. During their life, parasites encounter changes in the environment from relatively alkaline, sugar- and amino acid-rich, to acidic, fatty acid- and amino acid-rich environments. *Leishmania* cells have developed mechanisms of adaptation that favor utilization of amino acids. We have identified a cascade of two identical adjacent genes in the reverse strand of *Leishmania donovani* chromosome 10 (*LdAAP24*) that due to alternative *trans* splicing encode for two distinct membrane transporters; one is 18 amino acid shorter in the N-terminus (LdAAP24a) than the other (LdAAP24b). LdAAP24a translocates alanine only whereas LdAAP24b both proline and alanine. Unlike all other organisms, *Leishmania* (and all members of the Tyrpanosomatid family) maintain a large cellular pool of proline that, together with the alanine pool, serve as alternative carbon sources as well as reservoirs of organic osmolytes. LdAAP24a and b are the sole suppliers for the intracellular pool of proline and contributes to the alanine pool.

The *E. histolytica* methyl line binding protein EhMLBP serves as a bridge between environmental stress response and epigenetic regulation.

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Background: Adaptation to environmental stress is a key process that allows the unicellular parasite *Entamoeba histolytica* to survive in its human host. We previously characterized EhMLBP as an essential protein for the growth and the virulence of the parasite. EhMLBP binds to methylated repetitive DNA, and is one of the core proteins of the parasite's epigenetic machinery. Here, we show that EhMLBP and heat shock proteins have common properties. EhMLBP is induced by heat shock and its expression is regulated by a heat shock element binding site that is located in its 5' non-coding region.
Results: Following heat shock, EhMLBP displays an enhanced recruitment to the reverse transcriptase of a long interspersed nucleotide element (LINE) DNA. Constitutive overexpression of EhMLBP leads to an enhanced transcription of RT LINE and protects trophozoites against heat shock and reduces protein aggregation. Furthermore, upon heat shock EhMLBP is able to bind polyubiquitinated proteins.

The protective function of EhMLBP to heat shock and its ability to bind polyubiquitinated proteins is lost in trophozoites that overexpress a mutated form of EhMLBP which is devoid of its heat shock domain. Following heat shock, the perinuclear localization of EhMLBP in control trophozoites is replaced by an even distribution within the nucleus and with the appearance of cytoplasmic vesicles. The disappearance of these vesicles in cyclohexamide-treated trophozoites suggests that these vesicles correspond to stress granules. In addition, we will present first evidences that EhMLBP binds RNA, a function that may be related to its role as a stress granule protein.
Conclusions: To the best of our knowledge, this is the first report of a methyl DNA binding protein that plays a protective role against heat shock.

14:15 – 16:15 Session 3: Tropical Medicine

14:15 – 14:35 ***Leon Gilead*** **- Hansen's disease in Israel: An update**

14:35 – 14:50 ***Roy Faiman, Ibrahim Abbasi, Oscar Kirstein, Moshe Torem and Alon Warburg*** **- A newly emerged cutaneous leishmaniasis focus in northern Israel and a promising means of vector control -**

14:50 – 15:05 ***Michal Solomon, Shmuel Benenson, Sharon Baum and Eli Schwartz*** **- Tropical skin infections among Israeli travelers**

15:05 – 15:20 ***Eran Kopel, Enbal Marhoom Yechezkel Sidi and Eli Schwartz*** **- Successful oral therapy for severe falciparum malaria: The World Health Organization criteria revisited**

15:20 – 15:40 ***Jacob Moran-Gilad*** **- Waterborne infections and mass gatherings: The case of rapid laboratory diagnosis of leptospirosis**

15:40 – 15:55 ***Orli Sagi, Elena Sadikov and Shalom Ben Shimol*** **- Evaluation of enzyme-linked immunosorbent assay (ELISA) and Western blot analysis (WB) for Echinococcosis serodiagnosis**

15:55 – 16:15 ***Amit Hananel, Shimon Gross, Marcelo Fridlender, Vladimir Hurgin, June Kopelowitz, Oded Babai, Lital Levi and Zvi Greenberg*** **- Providing a comprehensive solution for the diagnosis of gastrointestinal protozoan parasites – Molecular-based and antigen detection tests**

Hansen's disease in Israel – An update
Leon Gilead

The Israeli Hansen's Disease Center, Department of Dermatology, Hadassah University Medical Center, Jerusalem

Hansen's disease (previously erroneously named 'Tsarath' in Hebrew and 'Leprosy' in Latin) is a chronic, granulomatous, infectious disease, targeting skin and peripheral nerve system and caused by the bacterium *Mycobacterium leprae*. Following the discovery of effective antimicrobial agents and the development of an effective treatment protocol, the World Health Organization (WHO) has declared a campaign for the elimination of leprosy as a public health problem by the year 2000. This campaign, though unsuccessful, did succeed in eliminating awareness to the continued presence of the disease. According to the WHO data the prevalence and incidence of the disease is declining nowadays, but leprologists are worried that changes introduced into the treatment protocol and health policy, as well as data from non WHO sources indicate the disease is about to make a comeback. In spite of the supposedly regular infectious disease nature (with a special course and symptoms), even today those suffering from the disease continue to be subjects of a horrible and completely unjustified stigma. A delay in diagnosis and administration of proper care and medication may allow, even today, for the development of severe peripheral motor and sensory nerve damage which may result in deformation and disability. The early diagnosis of the disease is challenging for the clinician especially because of the diverse and at times subtle clinical manifestations. This is especially true in developed countries like Israel, in which the disease prevalence is extremely low, and it is therefore important to raise awareness to its continued presence. There are currently more than 200 registered patients in Israel, suffering from the disease and or its consequences, most of which suffer from neurological damages and disability. Israel is the target of immigration from Hansen's disease endemic countries and every year between 5-10 new cases are discovered (this year we have 4 new cases thus far). It is a certainty that there are in Israel additional, yet undiagnosed patients who may suffer severe damage due to delayed or wrong diagnosis and treatment. All Hansen's disease patients in Israel are treated at the Israeli Hansen's Disease Center**,** which specializes in diagnosing, treating and caring for these patients. The center also initiates contact surveys, guidance to medical personnel caring of patients and seminars to promote awareness to the disease and the ways of its diagnosis.

A newly emerged cutaneous leishmaniasis focus in northern Israel and a promising means of vector control
Roy Faiman[1], Ibrahim Abbasi[1], Oscar Kirstein[1], Moshe Torem[2] and Alon Warburg[1]

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In 2007 fifteen cases of cutaneous leishmaniasis (CL) from Sde Eliyahu (pop. 650), a Kibbutz in the Beit She'an valley of Israel, were reported to the Israel Ministry of Health. During 2008-2011 reported cases from the Kibbutz totaled 99 (15.2% of the

total population). The majority of the cases reside along the perimeter fence of the Kibbutz (>90%). Direct parasite isolates, PCR and RFLP from skin lesions indicated the causative agent was *Leishmania major* (Kinetoplastida: Trypanosomatida). This parasite is transmitted by the sand fly *Phlebotomus papatasi* (Diptera: Psychodidae) which constitutes up to 95% of the catch along the perimeter. Marking sand flies in-situ indicated directionality in their foraging behavior, entering the Kibbutz from the nearby surroundings. PCR screening for *Leishmania* ribosomal DNA (ITS1) of over 1,200 females trapped within the Kibbutz resulted with an 18% mean infection rate (range: 2.5-30%), increasing toward the end of summer. Gut removal and microscopic examination of a sample of sand flies from Sde Eliyahu enabled us to isolate, type and culture local *L. major* parasites for a transmission cycle study currently in progress. Sampling the rodent fauna in the Kibbutz vicinity revealed absence of the documented reservoir *Psammomys obesus* (Rodentia: Gerbillinae) within several km radius. The Social (=Gunther's) vole *Microtus guentheri* (Rodentia: Microtinae) is abundant throughout the region, heavily infesting the agricultural fields closely surrounding the Kibbutz. A sample of 164 voles was collected from the surrounding fields and tested for *Leishmania* ITS1 by PCR; ear-tip tissue from 27 voles (16.5%) tested positive for *Leishmania*. Reverse Line Blotting resulted with *L. major* as the causative parasite. Smaller numbers of *Meriones tristrami* (Rodentia: Gerbillinae), found further away from the Kibbutz, were also found infected by PCR (14 of 27). Applying a 2 m high sand fly-proof Fence from Vestergaard Frandsen onto the Kibbutz perimeter fence where sand fly numbers were highest resulted in a significant reduction (85%) of sand flies inside the protected houses behind the fence compared to houses not protected by it. To the best of our knowledge, this is the first report implicating *Mic. guentheri* and *Mer. tristrami* as potential reservoirs of *Leishmania*. With the widespread co-distribution of both vole and *P. papatasi* sand flies in Israel and the region, the potential for emerging foci of CL may increase, necessitating novel and effective control measures such as described herein.

Tropical skin infections among Israeli travelers

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Infectious skin disorders are common dermatologic illnesses in travelers. Knowledge of post-travel-related infectious skin disorders, will allow effective pre and post-travel counseling. All cases of returning travelers seen in our center, seeking care for infectious skin diseases were included in this study. For a comparison, data on returned travelers with non-infectious skin diseases and of healthy travelers, who had a pre-travel consultation in our institution, were also analyzed**.** Altogether, skin-related diagnosis was reported in 540 ill returned travelers, among them 286 (53%) had infectious skin diseases. Tropical skin infection was diagnosed in 64% of the infectious cases. Travelers returning from Latin America were significantly more ill with tropical skin infections than those traveling to Asia and Africa, The most

common diagnoses were: cutaneous leishmaniasis, myiasis and cutaneous larva migrans. In conclusion, tropical skin infections are common among Israeli travelers especially among those who visited Latin America.

Successful oral therapy for severe falciparum malaria: The World Health Organization criteria revisited

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Background: Severe malaria may be manifested with rapidly evolving, life-threatening conditions such as coma, metabolic acidosis, severe anemia, hypoglycemia, acute renal failure, or acute pulmonary edema. The World Health Organization definition for severe malaria is broad and includes additional criteria, each of which is sufficient to define it, such as hyperparasitemia or failure to feed.
Case Reports: We report a successful treatment of severe falciparum malaria in a non-immune adult patient with 30% parasitemia treated with the 6-dose oral regimen of artemether plus lumefantrine combination therapy alone. We have also retrospectively searched our tertiary center's database for similar cases and we have found two additional severe malaria cases, resolved uneventfully with oral regimen.
Conclusion: These cases might indicate a need to specifically address the definition of severe and complicated malaria in non-immune patients either in designated guidelines or as an explicit addition to the historical World Health Organization criteria.

Waterborne infections and mass gatherings: The case of rapid laboratory diagnosis of leptospirosis

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Waterborne infections are a major risk associated with sports activities that take place in aquatic environments. Therefore, the planning of mass events such as international sports competitions or Olympic Games should involve a risk assessment for the occurrence of waterborne infections and a proper risk management strategy. Leptospirosis is a potentially severe zoonotic infection that may be acquired following exposure to water contaminated by animal excreta. Leptospirosis is thus a potential sports-related waterborne infection, as demonstrated in the past by explosive outbreaks related to white water rafting. The diagnosis of leptospirosis relies on serological methods such as screening IgM ELISA and confirmatory microscopic agglutination test (MAT) which have many limitations. Molecular microbiological methods are increasingly being used for clinical diagnosis of infections. Despite significant progress in that field, there is currently no molecular assay that is considered a 'gold standard' for the diagnosis of leptospirosis. Several assays have

been developed over recent years by different groups, but none have gained wide acceptance. A molecular assay for diagnosis of leptospirosis should preferably be able to diagnose infection early in the course of illness through detection of even a low level of spirochaetemia and also able to provide a high degree of assurance, especially if utilised in the context of mass gatherings. Since non-pathogenic species such as *L. biflexa* are potential laboratory contaminants, molecular assays should not only detect *Leptospira spp*. but also differentiate between pathogenic and non-pathogenic genomospecies. Finally, the application of PCR on blood requires adequate quality to control due to potential reaction inhibition. A new molecular assay is currently being developed at the Microbiology Services of the United Kingdom Health Protection Agency (HPA) using real-time PCR chemistry. The assay will utilise two independent genetic targets and based on optimisation performed using pyrosequencing approaches, is expected to be able to differentiate between pathogenic and non-pathogenic *Leptospira spp*. if applied on blood samples obtained from suspected cases. Preliminary results of the analytical validation suggest high specificity and sensitivity to a single DNA copy. Clinical validation of the assay is underway and there is an opportunity for interested laboratories to participate in field validation studies in collaboration with the HPA. The assay is expected to be implemented in the future for diagnosis of sporadic autochthonous and travel-associated leptospirosis and possibly as a response measure to investigate water sports-related clusters of illness. Future validation for environmental testing may also be considered.

Evaluation of enzyme-linked immunosorbent assay (ELISA) and Western blot analysis (WB) for Echinococcosis serodiagnosis

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Introduction: Human echinococcosis (hydatid cyst disease), a zoonotic disease caused by a tapeworm, is usually diagnosed by serology and imaging techniques. Serologic tests sensitivity is related to cyst location, integrity, and vitality of the larval cyst. False negative serologic test results do not rule out echinococcosis, since some cyst carriers do not have detectable antibodies. False-positive reactions may occur in persons with other helminthic infections, cancer, and chronic immune disorders. Enzyme-linked immunosorbent assay (ELISA) is a sensitive test for detecting antibodies in the serum of patients with cystic disease; sensitivity rates vary from 60% to 90%, depending on the characteristics of the cases. Western blot (WB) analysis became widely accepted in recent years, as this technique allows the separation of antigens through protein electrophoresis, thus providing specific diagnosis. Our main objective was to determine specific serology validity for diagnosis of echinococcosis with ELISA and WB techniques used separately and in parallel.

Methods: Serum samples taken from 100 patients were evaluated with ELISA method (r-biopharm). 56 out of 100 serums were evaluated for echinococcosis with WB (EUROIMMIN) technique.

Results: (preliminary): 41 of 100 serums (41%) were tested positive for echinococcal antibodies by ELISA. Of these samples, 26 (63%) were positive for 7kDa antigen (highly specific), 2 (5%) were positive for 24-26/16-18kDa antigen and 13 (32%) were tested negative (4/41). 15 samples, which were tested negative for echinococcal antibodies by ELISA were all negative in WB.

Conclusions: Our results indicate that the best available serologic diagnosis is obtained by using combination of tests. ELISA for initial sensitive screening of sera and confirmation of reactivity by demonstration of specific echinococcal antigens by immunoblot assays.

Providing a comprehensive solution for the diagnosis of gastrointestinal protozoan parasites – molecular-based and antigen detection tests

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Diarrheal diseases are extremely common in developed and developing countries and are major causes of morbidity and mortality, affecting millions of individuals each year. Protozoan parasites are known as abundant etiologies of gastrointestinal (GI) clinical symptoms. The most common intestinal protozoan parasites infecting humans worldwide are considered to be *Entamoeba histolytica*, *Giardia lamblia*, *Blastocystis hominis, Dientamoeba fragilis* and *Cryptosporidium spp.* Accurate diagnosis is crucial to exclude other possible etiologies and to allow appropriate treatment as soon as possible. Stool testing at the microbiology laboratory, especially by microscopy or culture, is currently a complex, time consuming and laborious process that demands highly qualified personnel and application of a wide range of techniques. As a result, workload, lab space and turnaround time are high and costly. Savyon's approach is to provide a comprehensive solution to the need to diagnose protozoan parasites in a cost-effective manner. We offer two methodologies that may work either together in a complementary way or as alternative to each other, according to the specific lab preferences. One methodology takes the advantage of using our proprietary NanoCHIP® technology, a molecular based electronic microarray test with profound multiplexing capabilities. The NanoCHIP involves extraction of DNA from stool samples, carrying out PCR, and loading the resulted amplicons onto the NanoCHIP platform. The amplicons are hybridized to specific oligonucleotides that were previously addressed to particular array locations. The target amplicons are detected by colored probes and the results are received directly following automatic data analysis. The other methodology utilizes the ELISA for antigen detection as a two-stage process. The first stage, an "all-in-one" assay, is intended to screen for all the mentioned parasites without distinction, when the multiplexing is achieved in one well, while the second stage is for separate detection of the specific etiology in different wells. Both approaches, the NanoCHIP and ELISA are adapted as cost-effective tests for screening purposes, require no specific skills, minimal hands-on time, improve the lab workflow and results are received within the same working day time frame. The comprehensive approach is believed to improve the diagnosis of protozoan parasites in GI symptomatic patients, enabling various types of laboratories to adopt their own preferred methodology.

14:15 – 16:30 **Session 4: General Parasitology**

14:15 – 14:30 ***Ma'ayan Papini*** ***and Charles L. Jaffe*** **- What's apoptosis got to do with it?**

14:30 – 14:45 ***Monica L. Mazuz, Richard Haynes, Varda Shkap and Jacob Golenser*** **- Neosporosis: successful treatment with artemisone**

14:45 – 15:00 ***Dalit Talmi-Frank, Aysheshem Kassahun, Carla Maia, Iva Rohousova, Roni King, Arie Zackay, Charles L. Jaffe, Alon Warburg, Asrat Hailu and Gad Baneth*** **– Epidemiological study of Leishmania infection in farm animals in northern Ethiopia**

15:00 – 15:15 ***Adi Moncaz, Oscar Kirstein, Roy Faiman and Alon Warburg*** **- Trapping phlebotomine sand flies using different attractants**

15:15 – 15:30 ***Oscar Kirstein, Roy Faiman, Adi Moncaz, H. Gueta, A Gurarie and Alon Warburg*** **- Studies on the behavior and control of phlebotomine sand flies (Diptera: Psychodidae) using experimental houses**

15:30 – 15:45 ***Yifat Guthmann, Eyal Klement, Dalit Talmi-Frank, Roni King, Tamar Yeger, David Meir, Laor Orshan and Gad Baneth*** **- Leishmania major in wildlife in Israel: animal hosts, infection rate, geographic distribution, and correlations with climate and seasonality**

15:45 – 16:00 ***Dalit Talmi-Frank, Yifat Guthmann, Roni King, Laor Orshan, David Meir, Tamar Yeger, Charles L. Jaffe and Gad Baneth - Leishmania tropica*** **in the rock hyrax in Israel: From epidemiology to pathology**

16:00 – 16:15 ***Sagiv Ben-Yakir*** **- The use of medical leeches for animal benefit**

16:15 - 16:30 ***Gad Baneth, Alina Sheiner, Osnat Eyal, Shelley Hahn and Dalit Talmi-Frank - Hepatozoon felis*** **infection in**

domestic cats – morphologic description and genetic characterization

What's apoptosis got to do with it?

Ma'ayan Papini and Charles L. Jaffe

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CDKs are a sub group of serine/threonine protein kinases that play a pivotal role in the cell cycle progression, transcription, differentiation, cell death and more. Due to their involvement in many vital cellular functions, and role in cancer and other diseases, CDK specific inhibitors have been developed. Paullones are one class of CDK inhibitors that block CDK1, CDK2 and GSK-3 activity. Several paullone derivatives have also been shown to kill *Leishmania* and African trypanosomes. Induction of apoptosis by three structural similar compounds showing high activity against *L. donovani* (GI_{50}: A=1.0, B=1.83 and C=0.76 μM) and *T. b. rhodesiense* (GI_{50}: A=0.9 μM and C=0.6 μM) was examined using *L. donovani* promastigotes. All three compounds induce programmed cell death as indicated by analysis of several cell markers including externalization of phosphatidyl serine, mitochondrial depolarization, caspase 3/7 activation, and elevation in promastigote sub G0/G1 phase, however the kinetics of apoptosis was different for each compound tested. Compounds A and C induced apoptosis as indicated by phosphatidyl serine externalization in up to 49.6% of treated promastigotes after 72 hours. Loss of mitochondrial potential was displayed in 83.13 - 87.58% of the treated parasites, as well as caspase 3/7 activation and elevation in sub G0/G1 phase compared to control parasites. Parasites treated with compound B showed some characteristics of apoptosis but to a lesser extent. Affinity chromatography using a small molecule inhibitor bound to a resin followed by LC MS-MS was used to purify and identify the putative target of these compounds as elongation Factor 1 (EF-1). Taken together, these findings indicate that apoptosis is the main mechanism by which these paullone-derived compounds kill *Leishmania,* and further investigation is needed to determine whether these compounds also act on *T. b. rhodesiense* in a similar fashion.

Neosporosis: successful treatment with artemisone

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Neospora caninum is an obligatory intracellular apicomplexa protozoan parasite of worldwide distribution that infects a wide range of livestock and domestic animals, including cattle. Neosporosis has global clinical and economical impact, mainly in the cattle industry. *N. caninum* is the major cause of abortion in dairy and beef cattle worldwide. In Israel more than 45% of dams carry specific antibodies and 18% of abortions were associated with *N. caninum* infection, as confirmed by serological and molecular assays in dams and aborted fetuses. Currently, there is no chemotherapy for bovine neosporosis that has been shown to be safe and effective. This publication describes the significant beneficial *in vitro and in vivo* effects of artemisone on *N. caninum* infections. Artemisone is a recent semi-synthetic 10-alkylamino artemisinin, that is superior to other artemisinin derivatives in terms of its higher anti malarial and anti toxoplasmosis activities, its tolerance *in vivo* and lack of detectable neurotoxic potential, improved *in vivo* pharmacokinetics and metabolic stability. Low μM concentrations of artemisone inhibited *in vitro N. caninum* development, and reduced the number of infected cells and the number of parasites per cell. In the *in vivo* gerbil model, a non-toxic dose prevented typical cerebral symptoms, in most animals. *N. caninum* specific PCR of brain of treated animals was negative. Surviving gerbils produced high specific antibody titer and were protected against a challenge. In conclusion, artemisone exhibits pronounced *in vitro* and *in vivo* inhibitory activity against *N. caninum* proliferation and is a promising candidate drug for prevention and treatment of neosporosis.

Epidemiological study of *Leishmania* infection in farm animals in northern Ethiopia

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Introduction. Visceral leishmaniasis (VL) is caused in Ethiopia by parasites of the *L. donovani* complex. It is responsible for severe morbidity in more than 4,000 people annually. The worst affected region which accounts for more than 60% of the reported VL cases is northern Ethiopia (NE) bordering Sudan and Eritrea. Human infections are frequently associated with HIV/AIDS. In NE and in Sudan *Phlebotomus orientalis* (*Ph. orientalis*) sandflies, widespread in acacia woodlands and black cotton soil, have been implicated in transmitting the infection, whereas in southern Ethiopia, *Ph. martini* and *Ph. ciliae* are implicated as the vectors of the disease. Although VL in Ethiopia is frequently found in agricultural regions where people live in close contact with farm animals, epidemiological studies to investigate the possible role of animals in the transmission of the disease have not been conclusive.
Materials and Methods. Blood samples were collected at 3 different locations in NE including Humera, Sheraro and Addis zemen. A total of 636 animals were been sampled including goats (n=278), sheep (n=168), cows (n=117), dogs (n=38), donkeys (n=23), and camels (n=2). Samples were analyzed using real-time PCR and

HRM analysis for a kDNA fragment and for the rRNA ITS1 locus. All PCR products were verified by sequencing. Statistical analysis was done using Fisher's P test and Pearson chi-square test.
Results. Altogether 636 animals were sampled. The total infection rate was 5% (33/636) using the kDNA real-time PCR method, while ITS1-HRM was far less sensitive and detected 9/636 (1.5%) positive samples. All samples positive by the ITS1 PCR were also positive by the kDNA PCR. Positive animals included cows, donkeys, sheep and goats. Infection rates varied slightly between different animals species ranging between 3.5-8.5% without statistically-significant differences. Only 1 dog was positive on a conjunctival sample. Positive samples were confirmed as *L. donovani* using the specific CpB PCR and RFLP for distinction between *L. infantum* and *L. donovani*. Infection was more widespread in Humera where 10% of the animals were positive by kDNA PCR, whereas only 0.5% and 0.8% of the animals in Sheraro and Addis Zemen, respectively, were found positive.
Conclusions. This study provides evidence for *L. donovani* infection in farm animals in areas endemic for *L. donovani* in people. The possible role of animals in *L. donovani* infection, usually considered to have an anthroponotic transmission, is currently unknown. Animals may be exposed to infection in parallel to humans, or could have a role in the transmission of infection, possibly as a reservoir for the disease. Animal involvement of *L. donovani* infection in Ethiopia should be further evaluated by additional methods including xenodiagnosis and sandfly blood meal analysis.
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Trapping phlebotomine sand flies using different attractants

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Current trapping methods employed for monitoring sand fly populations include passive as well as baited traps. While passive trapping is simple, inexpensive and maintenance free, its efficiency is rather low. For example, in North East Ethiopia and the Judean Desert, mostly male sand flies were captured on horizontal sticky traps while females were usually absent. CDC traps baited with dry ice are considered efficient for trapping unfed female sand flies. However, dry ice is cumbersome to transport and is mostly unavailable in remote areas. Therefore, we experimented with fermenting sugar solutions as a source of CO_2 for attracting sand flies to different types of traps. In initial studies we used 8 CDC traps, 4 of which were baited with dry ice and 4 with sugar/yeasts mixture (120g/12g in 1.5 lit tap water). A total of 521 sand flies were trapped. Of these 389 (75%) were trapped by the traps baited with dry ice (97 flies/trap/night) and 132 (25%) were trapped, in the sugar/yeast baited traps (33 flies/trap/night). The predominant species was *Phlebotomus. papatasi* (86% in dry ice, and 70% in sugar/yeast baited traps). Male/female ratio for *Ph. papatasi* was approximately equal in both trap types. In a follow up experiments, we used

horizontal sticky traps made of plastic boards (Polygal 60x60 cm) with their upper surface smeared with castor oil that were placed ~15cm above ground on a low metal frame. Four attractants were tested: Dry ice, sugar/yeast solution, ethanol 10% solution, and chloroform 3% tested against non-supplemented sticky trap. Traps were left over night and Sand flies were collected using fine forceps and placed in detergent solution for transport to the laboratory. A total of 2396 sand flies were trapped during a 6 nights experiment. Trap yields were: 258 sand flies/trap/night for dry ice, 116 flies/trap/night for sugar/yeast solution, 10 flies/trap/night for ethanol, 9 flies/trap/night for chloroform bated yeasts and 6 flies/trap/night for non-bated Sticky Traps. *Ph. papatasi* was by far the most abundant species (93%), and the male/female ratio was similar in both dry ice and sugar/yeast baited traps (64% and 66% males respectively). Conclusions were that sticky traps baited with sugar/yeast solution can provide a readily available, inexpensive and efficient means for sampling sand flies in remote areas.

Studies on the behavior and control of phlebotomine sand flies (Diptera: Psychodidae) using experimental houses

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Epidemiological studies show that Cutaneus Leishmanianis (CL) and Visceral Leishmaniasis (VL) in the Middle East are re-emerging as important public health problems in areas that were previously disease free (Jaffe et al., 2004). The control of phlebotomine sand flies (Diptera: Psichodidade) is notoriously problematic because the breeding sites of the immature stages are mostly unknown and usually inaccessible. Therefore, larval source reduction, which is the main approach in mosquito control, is impractical in the case of sand flies (Alexander and Maroli, 2003). Understanding the adults' sand flies foraging behavior in and around human habitation is an important clue before any control intervention. To improve the understanding on how foraging sand flies precede on flat and vertical surface, sticky tarps were places on a vertical wall showing an upward direction of the sand flies from the ground. To study the approach patterns of sand flies before entering windows we used sticky traps surrounding the windows. Preliminary studies showed that flies approach windows by hopping on the external walls from all directions. However, more of them approach from below and significantly fewer from above the window. Based on such data, we installed external shelves under windows and smeared their undersides with castor oil. In preliminary experiments, conducted in inhabited houses, the number of sand flies entering homes was reduced by 50% compared to baseline catches and neighboring control houses ($P<0.05$). However, actual results using shelves in the experimental house were less encouraging showing that flies avoid landing on the underside of the shelves and simply fly over or around it. Conclusions are that sand flies normally advance in short flights close to the ground. When they encounter a vertical obstacle like a wall and are attracted by CO_2

or other cues emanating from openings such as windows, they move up in similarly short flights. Therefore, insecticide-sprayed walls or vertical nets should be effective for controlling sand flies approaching human habitation.

Leishmania major in wildlife in Israel: animal hosts, infection rate, geographic distribution, and correlations with climate and seasonality

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A continuous and gradual increase in the incidence of cutaneous leishmaniasis (CL) due to *Leishmania major* has been reported in Israel over the last 20 years. The goal of this study was to determine the current status of *L. major* infection in wildlife with respect to potential hosts and reservoir animals, geographic distribution, climate and seasonality. The study included trapping and sampling of wildlife mammals by the Israel Nature and Parks wardens in 14 locations throughout Israel from July 2008 to June 2011. These included 5 locations known as human foci of *L. major* infection and 5 locations in which this disease has not been reported. *L. major* infection was determined by detection of the parasite's DNA by Real-Time PCR and High Resolution Melt analysis confirmed by DNA sequencing. In all, 1494 animals of 28 different wild mammal species were trapped and sampled, including the common reservoir *Psammomys obesus*. Distribution maps and attribution to climate regions were carried out using the Geographic Information System (GIS) software ARCGIS v9.3® (ESRI). *L. major* infection was recorded in 14 of the 28 species examined with the highest infection rate in *Psammomys obesus* (22/45; 49%); high prevalence was also recorded in other rodent species such as *Meriones crassus* (3/7; 43%), *Paraechinus aethiopicus* (3/9; 33%), *Gerbillus dasyurus* (7/30; 23%), *Erinaceus concoclor* (3/13; 23%), and *Meriones tristami* (8/59; 14%). Wild mammals infected with *L. major* were found in 12 locations across Israel. The highest prevalence was recorded at Revivim (54%), Urim (53%), and Qziot (33%). The infection rate in the arid climate was significantly higher than in the semi-arid and the Mediterranean climates (p=0.0001), moreover, more infected species and higher infection rates were found in the arid climate zone. *L. major* infection in the examined mammals was found continuously throughout all four seasons. This study has detected *L. major* in a large number of hosts previously not know to be infected with this parasite in Israel. The high infection rates detected in the hedgehogs *Erinaceus concoclor* and *Paraechinus aethiopicus*is is of special interest for further studies. The high rate of infection in some hosts and the continuity of infection throughout the whole year with high risk of infection in the arid climate are in agreement with the prevalence of infection in humans in the same areas.

Leishmania tropica in the rock hyrax in Israel: From epidemiology to pathology

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Introduction: Cutaneous leishmaniasis (CL) caused by *Leishmania tropica* is an important public health concern in Israel during the last decade. The disease has been reported in several foci including central as well as northern Israel. Several studies have shown that rock hyraxes (*Procavia capensis*) found in human foci of CL in Israel are infected with *L. tropica*. Infected hyraxes are suspected as reservoirs for human infection, however, data is lacking on the pathogenesis of infection in the hyrax, and the ability of naturally infected hyraxes to sustain long-term infection and thus serve as competent reservoirs for CL. For this reason, a multi-year study was designed to examine *L. tropica* infection in hyrax populations and sand fly activity in active human CL foci, as well as peripheral locations where human disease has not been recorded. In addition, the parasite's distribution in cutaneous and visceral hyrax tissues was determined using real-time PCR and high resolution melt (HRM) analysis, as well as tissue immunohistochemistry (IHC) and immunofluorescence (IFA). Infection was correlated with parameters such as season, gender, age and physical status.

Materials and Methods: The study included 5 foci where human CL is endemic, as well as 7 non endemic areas. Hyraxes were trapped and examined for external lesions and physical condition. They were anesthetized to collect blood, and than euthanized and dissected to collect samples from the ear pinna, snout, skin, spleen tissue, lymph nodes and bone marrow, as well as fetuses from pregnant females.

Results: Altogether, during a period of 28 months and 3 sand fly activity seasons, 223 rock hyraxes were captured at 12 different sites in Israel including endemic and non-endemic ones, and 1021 tissue samples were examined. Overall 42 of 223 (19%) hyraxes were positive for *L. tropica* ITS1 DNA using PCR-HRM. This was also confirmed by DNA sequencing**.** Positive hyraxes were found infected in all endemic foci for human CL, as well as in 2 of the 7 non-endemic sites. The hyrax infection rate ranged from 12% to 42% among the different locations. Hyraxes were consistently found to be *L. tropica* positive throughout 2008-2010 at the sites mentioned**,** with significantly higher infection rates in spring (March-May) compared to the summer months (June-August). Overall, positive hyraxes were trapped in all seasons, showing a continuity of infection regardless of the sand fly activity level. No statistical correlation was found between physical status such as the presence of dermal lesions or pregnancy, and infection with *L. tropica*. Infected tissues demonstrated using PCR-HRM, ICH and IFA included the ears, snout, spleen and skin.

Conclusions: Infected hyraxes were found at every study site in which human infection exists in addition to areas in a range of 10 - 20 km from the known endemic site. A continuity of high *L. tropica* infection rates were found in hyraxes throughout the year. Evidence of visceralizing infection was found in the hyrax spleen in addition to cutaneous infection in external tissues and blood. Amastigotes were demonstrated in the spleen, as well as in external tissues confirm the presence of the parasite in

these tissues. Together, these findings substantiate the rock hyrax as a reservoir animal for *L. tropica* in Israel, and suggest that hyrax infection surveys may predict the possibility of human *L. tropica* infection.

The use of medical leeches for animal benefit

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Application of medical leeches is used for solving blood related conditions in which blood is stagnated or in a stasis. Blood stagnation - a cessation of flowing or circulation of blood, or not running in a current or a stream, not flowing; motionless as can be seen in feline aortic thromboembolism, acute equine laminitis or canine aural hematoma. Blood Stasis - a slowing or stoppage of the normal flow of blood in an organ or a vessel in the body, such as slowing of the current of circulating blood in the arteries or the veins as can be seen in Polycythemia vera. Therapy involving medical leeches is indicated for solving blood stagnation and blood stasis in animals.
In the last decade we treated a few hundreds cats, horses and dogs by application of medical leeches in order to solve blood stagnation or blood stasis in a very good clinical success rate (92% returning to normal activity and functioning) if done within the first 72 hours. Post 72 hours the clinical success rate is reduced dramatically to less than 25%, and we conclude that time is an essence for a good clinical success rate for most medical leeching sessions.

***Hepatozoon felis* infection in domestic cats – morphologic description and genetic characterization**

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Hepatozoon species are apicomplexan parasites with a hematophagous arthropod final host and a vertebrate intermediate host. They are transmitted by ingestion of the final host containing mature oocysts by the intermediate host. The gamont stage of the parasite is found in leukocytes or erythrocytes of the intermediate host and infects the final host during the blood meal. Additional transmission pathways have been described in some *Hepatozoon* spp. including intrauterine transmission and carnivorism of the intermediate host by an intermediate host of a different species. More than 330 species of *Hepatozoon* have been described to date with 40 reported in mammals. A *Hepatozoon* parasite was reported for the first time from the blood of a domestic cat in India by Patton in 1908 and named *Hepatozoon felis domestici*. Since then, relatively little information has been published on hepatozoonosis in cats. Some authors have suggested that *Hepatozoon canis,* which infects dogs is responsible also for feline hepatozoononsis due to the morphological similarity of the gamont stage seen in the blood of dogs and cats. Several surveys have reported the detection of *Hepatozoon* DNA in the blood of cats by PCR and sequencing, with the depositionof

DNA sequence accessions in GenBank as *Hepatozoon felis*, however, these reports were not accompanied with descriptions of the parasite by microscopy of blood or tissue forms. Blood from 20 cats in which *Hepatozoon* gamonts were detected by blood smear microscopy at the Hebrew University Veterinary Teaching Hospital (VTH) during 2002 to 2011was collected and stored at – 80 C. In addition, tissue samples of cats in which *Hepatozoon* tissue forms were detected at necropsy, and in some cases also antemortem in the blood, were collected. Gamonts stages of the parasite from the blood of 20 cats and tissue stages from the skeletal muscles, myocardium and the lungs of 3 cats were measured and described. DNA was extracted from the blood and formalin-fixed, paraffin embedded tissues and PCR for a 358 bp fragment of the *Hepatozoon* 18S rRNA gene followed by sequencing of the amplicon were performed. Blood of several pregnant shelter queens brought to the VTH for neutering was also evaluated by PCR. Fetuses from these cats were frozen at -80 C and if the queen blood was found positive by PCR, fetal tissues PCR was carried out in order to detect possible intrauterine transmission. In addition, a molecular survey evaluated the presence of *Hepatozoon* DNA in the blood of 152 domestic cats collected from several areas in Israel. All PCR amplicons amplified from positive cats included in each part of the study were sequenced and compared by BLAST analysis to accessions present in GenBank. A phylogenetic analysis, which included several isolates from the blood and tissues of cats was carried out to compare these isolates to *Hepatozoon* species described in other animal hosts and in domestic cats. *Hepatozoon* DNA was amplified from the blood of 55 of 152 (36 %) of the cats surveyed. Infection was significantly more prevalent among cats with access to the environment, in comparison to strictly indoor cats ($p=0.00001$). In addition, *Hepatozoon* DNA was also amplified from all 20 cats included in the collection of cases detected by blood smear analysis at the VTH and from the paraffin embedded tissues of 3 cats in which *Hepatozoon* tissue forms were detected by histopathology. All the sequenced amplicons were 97-100 % identical to published sequences of *H. felis* except for 2 from cats detected at the VTH by blood smear microscopy that were closer (99 %) to *H. canis*. Fetuses from 3 queens were positive for *H. felis* in the lungs or amniotic fluid. By histopathology, tissue meronts in skeletal and cardiac muscles and in lungs were round and contained merozoites surrounded by a thick membrane separating them from the surrounding tissues, frequently without an evident inflammatory response.

In conclusion, this is the first study to combine the genetic analysis of *H. felis* with morphologic description of the parasite in the blood and tissues, and with an epidemiological survey. *Hepatozoon* infection appears to be common in the surveyed populations of Israeli cats and most infections appear to be sub-clinical. Meronts forms parasitize mostly muscular tissues but may also be found in other tissues such as the lung. The presence of parasite DNA reported for the first time in cat fetuses is suggestive of intrauterine transmission, as found also for *Hepatozoon canis* in the dog. No arthropod vectors have been described so far for feline heaptozoonosis. Genotyping of the feline isolates and phylogenetic analyses suggest that a unique *Hepatozoon* sp. infects cats which is genetically similar to the *H. felis* sequences deposited in GenBank from other locations in the world. However, the findings of two isolates which appear to be similar to *H. canis* suggests that *H. canis* may also infect cats, as postulated by some authors.