ABSTRACTS

(In alphabetic order according to the name of the first author)

A SINE repetitive element is involved in the epigenetic transcriptional silencing of the amoebapore gene in *Entamoeba histolytica*

Anbar M., Bracha R., Nuchamowitz Y., Florentin A., Persky M. and Mirelman D.

Department of Biological Chemistry, Weizmann Institute of Science, Rehovot

Transfection of amoebic trophozoites of virulent strain HM-1:IMSS with a plasmid construct containing 473 bp of the 5' flanking sequence of the amoebapore gene (*Ehap-a*), caused within 2 weeks, the complete transcriptional silencing of this gene. Sequence analysis of this 5' flanking segment revealed that the distal 140 bp were part of a neighboring short interspersed repetitive element (SINE1) that is actively transcribed from the antisense strand and is preceded by a thymidine rich (T-rich) region of 48 bp. Elimination of the SINE1 sequence from the plasmid construct prevented the silencing of the *Ehap-a* gene in new transfectants. *Ehap-a* silencing was achieved only when transfecting a truncated SINE1 element. Transfection with constructs which included either (i) the full length SINE1 element or (ii) a plasmid in which the 3' regulatory region of SINE1 was fused to the truncated 140 bp of the SINE1, or (iii) a plasmid (p5AP3:CAT) in which the chloramphenicol acetyltransferase (CAT) reporter gene, flanked at its 3' with a 3' actin regulatory region was fused to the truncated 5' SINE1 element, did not cause silencing of the *Ehap-a* gene. These results indicate that the trigger for silencing requires the presence of a truncated segment of the SINE1 element lacking any 3' regulatory sequences. Analysis of the 5' sequences that are required for expression of the CAT reporter gene revealed that both the 5' sequences within the SINE1 element and the 48 bp T-rich upstream region are essential to promote transcription. The transcription initiation site of this SINE1 element was identified by primer extension of the mRNA produced by a SINE:CAT fusion construct. The importance of the upstream T-rich region for transcription was also demonstrated for another SINE1 element which is adjacent to a proline rich gene. Transfection of the plasmid-less, *Ehap-a* silenced trophozoites (G3) with the p5AP3:CAT plasmid construct revealed a 5 fold increase in CAT expression indicating an activation of the SINE promoter in the G3 silenced trophozoites. RNA extracts from gene silenced cultures showed small amounts of short (~140 nt), single stranded molecules with homology to SINE1 but no siRNA. The molecular mechanism by which SINE repetitive elements appear to trigger the downstream silencing process is under investigation.

Ministry of Health guidelines for international travel – update 2005-6

Anis, Emilia and Daniele Goldmann

Department of Epidemiology and Infectious Diseases, Ministry of Health

The Department of Epidemiology and Infectious Diseases in the Ministry of Health periodically updates guidelines for international travel according to information received online from various international sources. In order to minimize differences in recommendations given by travel clinics of the Ministry of Health and travel clinics operated by others (HMO's, hospitals, private clinics), our department also issues frequent ad hoc notices for international travelers and online reports and news. The guidelines are published on the MoH internet site and the travel notices are e-mailed directly to the travel clinics. In cooperation with the Department of Health Promotion and Education and Nursing in Public Health in the MoH, we published a pamphlet for travelers including general as well as more specific recommendations regarding health precautions and preventive measures. Different issues updated recently or considered to be updated in the near future will be discussed.

Laboratory diagnosis of malaria

Assous, M.V., T. Cohen and E. Marva

Parasitology Reference Center, Government Central Laboratories, Ministry of Health, Jerusalem

The laboratory diagnosis of malaria is classically based on microscopy (examination of thin and thick blood smears) that represents the gold standard for the detection and identification of *Plasmodium* species. However, this procedure requires experienced and competent microscopists and is time consuming. When the parasitemia is very low (< 50 parasites/µl) or consists of only ring forms the diagnosis is much more difficult and sometimes restricted just to the genus level.

The rapid immunochromatographic antigen detection tests (RDTs) have become much more widely used in the last years. As compared with microscopy, their advantages are: better sensitivity, the rapidity of performing and the non-necessity of experienced personnel. They allow individual diagnosis (like microscopy), are well adapted to an emergency context and can differentiate between *Plasmodium falciparum* and other species. Nevertheless, they were reported to give non-specific positive reactions and false negative reactions particularly in case of *P. malariae* or *P. ovale* infections. It is recommended that use of RDTs should be coupled with microscopy; this is the case for the majority of hospital laboratories in Israel.

For malaria diagnosis, the use of molecular methods has been described for more than ten years. The major advantage of using PCR-based techniques is their ability to detect malaria parasites in patients with low levels of parasitemia and identify them at the species level. Thus they can be of great help in cases of discordance between microscopy and RDTs. Their disadvantages are the need of great expertise, their high cost and the impracticality to use them for individual diagnosis. For all these reasons, they are well adapted to state reference laboratories that centralize the cases. Since June 2004, a nested-PCR method was started at the Reference Center for Parasitology for all blood samples that are sent to us. As an example, a rare case of *P. ovale* will be presented. It demonstrates the utility of a reference laboratory that can help routine laboratories to diagnose questionable cases. Analysis of the results versus the epidemiological, clinical and therapeutical data will improve the management of this rare but potentially fatal infectious disease.

A study of visceral leishmaniasis in Israel and the Palestinian Authority using geographical information system (GIS)

G. Baneth[1], A. Amro[2], D. Strauss-Ayali[1,3], C.L. Jaffe[3], A. Warburg[3], S.S. Sawalha[2], K. Yossef[4], M. Hopp[4], J. Sztern[3] and Z.A. Abdeen[2]

[1] School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel.[2] Al Quds Nutrition and Health Research Institute, Al Quds University, Palestinian Authority.[3] Kuvin Center for the Study of Tropical and Infectious Diseases, The Hebrew University- Hadassah Medical School, Jerusalem, Israel. [4] Tel Aviv University, Tel Aviv, Israel

The objective of this study was to investigate the epidemiology of human and canine visceral leishmaniasis (VL) in Israel and the Palestinian Authority (PA) and the impact of geographical and ecological factors on its distribution. Databases of the human and canine cases of VL in Israel and the PA during 199O-2004 were constructed and analyzed for the year of diagnosis, geographic location and type of community (rural vs. urban). Sand flies trapping and previous collections from multiple locations throughout Israel and the PA were analyzed for species and sex.

In all, 176 people from the PA, of which 173 (98%) were under 10 years of age, and 14 people from Israel were reported with VL. Three cases of HIV/*Leishmania* co-infection from Israel are related to immigration from Ethiopia and may not be autochthonous. The majority of human VL cases were from the Hebron and Jenin districts in the PA. The incidence of VL in the Jenin district in children aged 0-2 years was 2.01/1,000 population and 1/1,000 in the Hebron district. One hundred and seventy seven dogs were detected with VL in Israel, 25% were from northern Israel and 75% from the central region. Seventy eight % of the dogs were from rural settings and 22% from urban locations. The number of infected dogs is probably underestimated due to lack of diagnosis and asymptomatic infection. An overlap was found between the regions in which canine and human VL were reported, however, no clinical human cases have been found in some of the active canine foci. Sand fly collections from 63 locations in Israel and in the PA indicated that *Phlebotomus tobbi*, *Ph. perfiliewi* and *Ph. syriacus*, considered vectors of *Leishmania infantum* in the eastern Mediterranean, were present in the disease foci. The distribution of these sand fly spp. roughly paralleled the geographic spread of VL. A significant statistical difference in the annual precipitation and patterns of land use was found between locations where suspected sandfly vectors were trapped vs. locations where non-vector sand fly spp. were trapped ($P< 0.05$). The spatial distribution of human VL in the West Bank was found to be significantly ($P< 0.05$) associated with a highly vegetated land use of olive trees and orchards, a relatively high annual rainfall of about 550 ml/year, low temperatures during October, moderate to high elevations, and high ground wind speed (ave. 5.2 m/sec).

Although previous studies have shown a considerable rate of asymptomatic exposure to VL in humans in Israel, the disease is mostly frequent among children in the West Bank. The reasons for this in the number of human cases in Israel and the PA should be studied further, and preventative measures should be implemented in disease foci.

Financial support: US-Israel Cooperative Development Research Program, Economic Growth. US Agency for International Development (Grant TA-MOU-00-C20-025)

Localized Onchocerciasis in Ethiopian immigrants to Israel

Barzilai, A., S. Baum, H. Trau and E. Schwartz

Department of Dermatology and Tropical Diseases Unit
Sheba Medical Center, Tel-Hashomer, Israel

Background: Onchcocerciasis is a chronic round-worm infection which involves the skin, subcutaneous tissue, lymph nodes and the ocular system. In the skin, it manifests itself as pruritus, dermatitis and onchocercomata. Involvement of the eye may lead to blindness. In Saudi Arabia, Yemen and East Africa, a localized type of onchocerciasis, called "sowda" exists.
Objective: To describe a series of 17 Ethiopian immigrants to Israel who have onchocerciasis.
Methods: Seventeen Ethiopian immigrants who presented to our hospital with clinical picture consistent with onchocerciasis were studied. Area of residency, onset of symptoms, the skin and ocular manifestations, laboratory evaluation as well as response to therapy were evaluated.
Results: Most of our patients (13/17) lived in the region of Quara, (north-west Ethiopia) and immigrated to Israel in 1999. Their ages ranged from 4 to 60 year-old. One patient was born in Israel presented with a pruritic rash when she was one year-old. In all of them the symptoms started after their arrival with an average incubation period of 2.5 years (1-4 years). All of them complained of localized pruritus involving the extremities, mostly the lower ones. The most common skin rash was chronic papular dermatitis (10/17) followed by lichenification (5/12) and post inflammatory changes (4/17). The extremities were involved in all cases. In 4 cases the trunk was also involved. Eye involvement was not detected. Eosinophilia and hyper immunoglobulin E were common. Snip test was positive only in 1 out of 4 cases in which it was performed, and 3/5 biopsies revealed chronic dermatitis with many eosinophils. Treatment with Ivermectin 200mcg/kg resulted in a temporary relief which lasted form 2 months to 2 years.
Conclusions: Onchocerciasis among Ethiopian immigrants seeing now in Israel is more localized disease, with a relatively long period of incubation and might be “Sowda”. This may be accounted by a hyper response of the immune system to the parasite. Transplacental transmission may occur. Physicians should be aware of this diagnosis in any patient who comes form an endemic area, complains of pruritus and has blood eosinophilia and hyper IgE.

Immunological responses and the pattern of disease in mice infected with transfected *Leishmania major* constitutively expressing active IL-1α

Bersudsky, M., A. Laban[1], R. N. Apte & J. El-On

Department of Microbiology and Immunology, Ben-Gurion University of the Negev, Beer-Sheva, Israel and [1]Department of Parasitology, Hadassah Medical School, Hebrew University, Jerusalem, Israel.

Immunity against leishmaniasis is mediated mainly by CD4+ T lymphocytes that function by secreting cytokines, which activate various effector mechanisms. Interleukin 1 (IL-1) represents one of the most pleiotropic pro-inflammatory cytokines required for normal regulation of Th1/Th2 responses. The aim of this study was to induce the expression of the inflammatory cytokine IL-1α by *Leishmania* parasites, and to determine their effect on the parasite development. *Leishmania* constitutively producing IL-1α was engineered, using 2 vectors, pLT1-Neo-IL-1α, and pX63Hyg-IL-1α. In the first group IL-1α was produced mainly by promastigotes and in the latter it was produced by both promastigotes and amastigotes, and remained unchanged after transformation and development in mice. Bone marrow macrophages infected with the transfected promastigotes produced a low level of IL-1α compared with undetectable IL-1α produced by the cells infected with the wild type. Stimulation of these cells with LPS induced similar IL-1α activity in the macrophages infected with either the transfected or the wild type parasites. The protection against the disease achieved in BALB/c mice by the transfected parasites (pX63Hyg-IL-1α) was superior to that obtained with the wild type. One month after infection, a nodule was demonstrated in 22% and 60% of the mice inoculated with transfected parasites and the wild type, respectively. This tendency continued for an additional 2.5 months, after which, the rate of infection was increased to 90% and 100% in these two groups, respectively. The present study suggests that during initial infection, the pathway of IL-1α production and its accessibility to the immunological cells might be important in the outcome of leishmanial infection.

***Entamoeba histolytica*: Epigenetic silencing of the amoebapore gene results in virulence - attenuated stable trophozoites**

Bracha, Rivka, Michael Anbar, Yael Nuchamowitz, Li Yan and David Mirelman

Department of Biological Chemistry, Weizman Institute of Science, Rehovot, Israel

Transfection of trophozoites of a virulent *E. histolytica* strain, HM-1:IMSS with a hybrid plasmid containing a genomic copy of the amoebapore gene (*Ehap-a*), including its upstream and downstream regulating elements, caused, instead of over-expression, a complete suppression of transcription of both, the episomal and chromosomal gene [Bracha et al. (2003) *Eukaryot. Cell*, 2, 295-305].. Nuclear run-on experiments revealed that gene silencing was at the transcription initiation level (TGS). Furthermore, removal of the plasmid from silenced trophozoites and long

term cultivation of cloned, plasmid-less trophozoites (termed G3) in the presence of inhibitors of DNA methyl transferases such as 5-Aza-Cytosine and Zebularine, or the histone deacetylase inhibitors Trichostatin A or butyrate, failed to restore the expression of the *Ehap-a* gene. Analysis of the 5' upstream fragment (470 bp) required for the triggering of gene silencing revealed that it contained the promoter region of the *Ehap-a* gene, a T-rich stretch, followed by a truncated SINE (Short Interspersed Element) that is transcribed from the antisense strand. Both, the T-rich stretch and sequences of the 5' SINE were essential for the transcription of the adjacent SINE element . RNA extracts from gene silenced cultures showed small amounts of short (~140 n), single stranded RNA molecules with homology to SINE1 but no siRNA. Chromatin immunoprecipitation (ChIP) analysis with an antibody against methylated K4 of histone H3 revealed a de-methylation of K4 at the domain of the *Ehap-a* gene indicating transcriptional inactivation (Anbar et al , Euk. Cell, in press)

The novel plasmid-less trophozoites (G3) were found to be non-virulent , they failed to kill mammalian cultured cells and did not induce liver lesions in hamsters even at inoculations of 10^6 trophozoites [Bujanover et al, Int. J. Parasitol. 33, 275-281,2003]. We have also demonstrated that in the absence of amoebapore expression, the trophozoites didn't cause amebic liver abscesses in the SCID mouse model . Nevertheless, the amoebapore-less trophozoites still caused some inflammation and tissue damage in infected human colonic xenografts [Zhang et al. Infect. Immun. 2004. 72: 678-83] Preliminary studies using intraperitoneal immunization of hamsters with the attenuated G3 strain showed that it evoked a significant humoral response which partially protected hamsters from challenge by the virulent *E. histolytica* strain. The virulence-attenuated trophozoites have the advantage of evoking an immune response to the same antigenic epitopes as of the virulent strain, and our recent success to silence an additional virulence gene may lead to a harmless, potential candidate for a live, oral vaccine against Amoebiasis.

The efficacy of paromomycin ointment (Leshcutan) combined with the immunomodulator Imiquimod on *Leishmania major* development *in vitro* in macrophages and *in vivo* in experimentally infected mice

El-On, J; R. Sneir & E. Bazarsky

Department of Microbiology and Immunology, Ben-Gurion University of the Negev and Soroka University Medical Center, Beer Sheva, Israel

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. Imiquimod (IMQ) (3M Pharmaceuticals, USA) is a novel immune-response-activating agent. It has recently been shown to act synergistically with anti-leishmanial compounds, given parenterally, in the treatment of CL, both in humans and in experimentally infected mice. The aim of this study was therefore to determine the efficacy of IMQ combined with Leshcutan (15% paromomycin sulphate (PR) and 12% methylbenzethonium chloride (MBCL) in soft white paraffin) as a topical application on CL lesions development *in vivo*, in experimentally infected mice. The efficacy of PR and MBCL combined with IMQ on *Leishmania major* amastigote development in macrophages *in vitro* was further analyzed.

After 2 and 4 days exposure of C3H mouse macrophages infected *in vitro* with *L. major* to PR at 25, 50 and 100µg/ml, an anti-leishmanial effect was observed that reduced the parasite survival index (PSI) to 65.5%, 38.8%, 25.1%, and 14.6%, 10.1%, 4.3%, respectively. IMQ at 5 and 10µg/ml reduced the PSI to 68.5% and 52.3%, on the second day of treatment that remained almost unchanged within additional 2 days of exposure to the drug. Combination chemotherapy of PR at 25, 50 and 100µg/ml and IMQ at either 5 µg/ml (PSI, 2^{ed} day = 51.9%, 22.1%, 18.8%; 4^{th} day = 16%, 13.4%, 5.8%) or 10µg/ml (PSI, 2^{ed} day = 43.5%, 33.9%, 17.8%; 4^{th} day = 10%, 7.9%, 5.1%), showed additive effect in all drug combinations examined. Similar results were also demonstrated with MBCL (0.1µg/ml, 0.5µg/ml) combined with IMQ (5µg/ml, 10µg/ml), showing PSI of 39.8%, 14.3% and 34.6%, 15.1% on the second day of treatment, respectively, vs. PSI = 60.5, 34.8 obtained with MBCL (0.1µg/ml, 0.5µg/ml) given alone. Further additional 2 days exposure of the cells to the drugs have not changed the results. A 10 days topical treatment, twice daily, with an ointment containing Leshcutan, either undiluted or diluted 1:5 in soft White paraffin + 1% IMQ, was as effective as Leshcutan given alone. A subsequent treatment with diluted Leshcutan and 1% IMQ, each given separately twice daily, did not change the results. Furthermore, 1% IMQ, either alone or containing the Leshcutan active ingredients diluted 1:5 (3gr PR and 2.4gr MBCL/100ml of 1% IMQ) barely affected the parasites load or the lesion size, mainly due to poor absorption and penetration of the drugs into the CL lesion.

Miltefosine (Impavido®) – A novel oral therapy of leishmaniasis

Anders, Gerlind

Zentaris GmbH, Germany

This presentation summarizes the published and ongoing studies on the efficacy and safety of Miltefosine in different forms of leishmaniasis. The therapeutic potential has been investigated in patients with visceral (VL), cutaneous (CL), mucocutaneous (MCL), and diffuse cutaneous leishmaniasis (DCL), and in addition, also in HIV-co-infected patients. Miltefosine has proved to be highly effective for the treatment of VL in India (95%), with similar efficacy and tolerance rates achieved in both, in hospital care (Phase-III-study) as well as in an out-patient setting (phase-IV-study). Studies of Old World and New World CL indicate the usefulness of Miltefosine in the therapy of CL. Ongoing trials in Bolivia (MCL) and Venezuela (DCL) are giving new hope to patients suffering from progressive facial disfigurement by achieving excellent initial responses. Only long term follow up will show final cure rates. In HIV-co-infected patients in Spain and in Ethiopa Miltefosine proved to be beneficial as well. In children down to an age of 2-3 years the efficacy and safety of Miltefosine has been demonstrated. The main side effects are transient, 1-2 days of nausea and vomiting in about 30% of patients. Contraception must be ensured in female patients of child bearing age. As a conclusion, Miltefosine has been tested extensively in VL and in different forms of New World CL. Its efficacy in Old World CL is presently being investigated. Miltefosine can be recommended for ambulant care.

Should chloroquine be laid to rest, or should it still be left in the pharmacopoeia for particular circumstances?

Ginsburg, Hagai

Department of Biological Chemistry, Institute of Life Sciences, Hebrew University, Jerusalem 91904, Israel

Chloroquine (CQ) has been the front line antimalarial drug due to its efficacy, low cost and scarce side effects, until resistance has evolved in the late 1950s. Although its use has been officially discontinued in most malaria-affected countries, it is in fact still widely used. Practical and pharmacological considerations indicate that it could be still used in semi-immune adults and that more efficient treatment protocols could be devised to treat even patients infected with CQ-resistant parasite strains. The improved use of an existing drug is clearly advantageous compared to the development and deployment of a totally new drug. The antimalarial activity of CQ is demonstrably pleiotropic. Hence, different mechanisms may underlie drug resistance. Most investigative efforts are centered on "reversers" that should ensure adequate accumulation of CQ to effective intracellular concentrations. However, additional and different mechanisms of resistance are hardly investigated. It is in these realms that additional enhancers of CQ action could be found to be used in drug combination.

Post-translational traslocation pathway in trypanosomes

Goldshmidt, Hanoch, Lilach Sheiner, Shai Uliel and Shulamit Michaeli

Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan

Both eukaryotes and prokaryotes traslocate secretory and membrane proteins across or into membranes. These proteins are translocated co-translationally by the SRP pathway, or post- translationally by the chaperone pathway, depending on the organism and specific features of the protein. RNAi silencing of a Signal Recognition Particle (SRP) protein (SRP54) in *Trypanosoma brucei* demonstrated that despite the inactivation of this pathway, signal-peptide containing proteins transversed the ER and were post-translationally modified, suggesting that there is an alternative pathway for protein translocation to the ER in these parasites (Liu et al., 2002, JBC *277*, 47348). In this study we present evidence for the existence of an active chaperone pathway in trypanosomes, and demonstrate its role in protein translocation across the ER. Sec71 protein which is essential for post-translational translocation in yeast was identified by bioinformatics and silenced by RNAi. The chaperone pathway is essential for growth in these parasites. However, unlike bacteria, the trypanosome signal-peptide containing proteins are not exclusively translocated by the chaperone pathway. All the signal-peptide containing proteins examined were translocated to the ER during Sec71 silencing, most probably by the SRP pathway. However, the most abundant GPI-anchored proteins of the procyclic parasites EP and GPEET were severely affected (both steady-state level and nascent synthesis) under silencing, suggesting that very abundant proteins maybe preferentially translocated via the chaperone pathway which is faster and more efficient compared with the SRP pathway.

Treatment of murine malaria by intranasal administration of Dihydroartemisinin in a permeation enhancing carrier

Golenser, Jacob[1], Judith H Waknine[1, 2], Biana Godin[2] and Elka Touitou[2]

Departments of Parasitology[1] and Pharmaceutics[2], Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem

Dihydroartemisinin (DHA) is considered to act by induction of oxidant stress. One mechanism suggests formation of radicals which induce non-specific damage. The second mechanism suggests specific inhibition of parasite Ca^{+2}-ATPase (SERCA). In support of the first theory, DHA in our laboratory was effective against eukaryotic cell lines, *Leishmania*, and bacteria, with ED_{50} values in the μM range. However, ED_{50} values obtained for *Plasmodium falciparum* were in the nM range, indicating a more specific mechanism. DHA is not commonly used to treat malaria due to its short plasma half life. However, direction of DHA to target organs may improve treatment. We investigated the efficacy of intranasal DHA administration from a novel carrier containing soft phospholipid vesicles. The combination of the nasal delivery pathway and a soft vesicular carrier (patent pending) may enable improved and sustained DHA absorption into the circulation. In vivo experiments were conducted using ICR female mice infected with *Plasmodium berghei anka*, a lethal model of malaria. The animals were treated under anesthesia with two daily doses of 5mg DHA/kg according to two schedules: (A) prophylaxis- starting 2 days before infection for a total of 6 days; (B) treatment- starting on day 2 after infection (parasites first detected) for 4 days. Mice were treated either by intranasal administration or by i.p. injection of the same DHA dose. All mice in the control groups succumbed to the disease: anesthesia and placebo (delivery carrier only) did not affect parasite development. In contrast, parasites were not detected in schedule A mice treated with intranasal administration of DHA in the novel carrier. Parasites appeared in 74% of mice treated with the same schedule by i.p. DHA injections. Seventy five percent of schedule B mice treated by intranasal DHA survived, in comparison with only 19% of mice treated i. p. The new dual approach used in this study, intranasal DHA administration from a permeation enhancing carrier, was effective in the prophylaxis and treatment of murine malaria and superior to i. p. injections.

Long-lasting helminthiasis in Ethiopian immigrants to Israel: *Strongyloides stercoralis*, hookworm and *Schistosoma mansoni*

Hassin, David, Miriam Kaufstein and Valery Istomin

Hillel Yaffe Medical Center, Hadera

Background: Helminthic infections are common in Ethiopia and rare in Israel. Helminthiasis in Ethiopian immigrants in Israel can teach us about the duration of persistence.
Methods: Ethiopian immigrants diagnosed with helminthiasis in an Israeli community hospital during 1994-2004 were studied.
Results: *Strongyloides stercoralis*, Hookworm and *Schistosoma mansoni* are the only persistent helminthic infections of long duration (>4 years) in Ethiopian immigrants in Israel. *S. stercoralis* infection was found in 12 adult patients, 5-24 years after immigration. Six of them had asthma. The clinical presentation at diagnosis was asthma exacerbation (4), gastrointestinal symptoms (3) and cachexia with lung infiltrates in an HIV infected patient (1). Four patients were asymptomatic. Eosinophilia was found in 7. Hookworm infections were found in 8 adult patients 4-12 years after immigration: 7 were asymptomatic, 4 had iron deficiency anemia one of which had severe symptomatic anemia. All were immunocompetent without any history of asthma. Four patients had eosinophilia. *S. mansoni* infections were found in 11 adult patients, 4-15 years after immigration: 8 patients were asymptomatic at diagnosis, 1 had nephrotic syndrome, 1 had carcinoma of the colon and 1 patient had acute appendicitis 15 years after immigration and the parasite was found in a periappendicular vein. Eosinophilia was present in 5.
Conclusions: The only persistent helminthic infections of long duration (>4 years) in Ethiopian immigrants are: *S. stercoralis*, Hookworm and *S. mansoni*. Asthma is associated with *S. stercoralis* infection. Ethiopian immigrants treated with steroids are at risk for hyperinfection caused by *S. stercoralis*. Hookworm infection can be a cause of iron deficiency anemia in Ethiopian immigrants. Anti helminthic empirical treatment should be considered in Ethiopian immigrants.

Pregnancy, breast feeding and travel vaccinations

Katzir, Michal and Michael Giladi

The Infectious Disease Unit and the Travel Advisory Clinic, Tel Aviv Sourasky Medical Center, Tel Aviv

The recent increase in international travel has included growing numbers of pregnant women. While extensive information exists on the efficacy and safety of vaccines in non-pregnant women, recommendations regarding pregnant women are less clear.
As a rule, it is considered best to avoid vaccinations unnecessary for the travel destination, particularly in the first trimester. Because of the increased risk for influenza-related complications, Influenza vaccine is recommended in any trimester for healthy pregnant women and pregnant women with high-risk medical conditions. Polio and diphtheria-tetanus (dT) vaccines have been safely administered to pregnant women, regardless of gestational age, in large population studies. The inactivated (IPV) rather than live oral polio vaccine (OPV) is preferred for the pregnant traveling woman. Although there are insufficient data on vaccines against hepatitis A (HAV), hepatitis B (HBV), meningococcal meningitis, and typhoid fever, these vaccines should be given to the pregnant traveler when the risks of infection outweigh the theoretical risks of immunization. When possible, recommendations for HAV and HBV vaccinations should be base on the pre travel serological status. No information is available on the safety of Japanese encephalitis vaccine during pregnancy. The latter should not be administered during pregnancy except when a woman must stay in a high-risk area. If not mandatory, travel should be postponed. Live attenuated Yellow Fever vaccine is required for visitors to countries in Africa and South America. Although concerns exist, no congenital abnormalities have been reported after administration of this vaccine to pregnant women. The vaccine should be administered to pregnant women only if travel to an endemic area is unavoidable and if an increased risk for exposure exists. If policy requires a yellow-fever vaccination certificate in countries where the disease is not a current threat, pregnant travelers are advised to carry a physician's waver. None of the above-mentioned vaccines, including Yellow Fever vaccine, is contraindicated during lactation.
In conclusion, risk to the developing fetus from vaccination of the mother during pregnancy is primarily theoretical. Recommendation for travel vaccination during pregnancy should be individualized based on the potential benefit and risk assessments.

Neurocysticercosis in Israel - A nation wide study

Kliers, Iris, Rivka Karplus, Moshe Gomori, Tal Zucker, Shoham Nir, Jihad Bishara, Israel Potasman, Yeheskel Sidi and Eli Schwartz

Introduction: Cyticercosis - a human infestation with *Taenia solium*, is endemic in less developed countries. Infection of the central nervous system results in neurocyticercosis (NCC), and is considered to be the most common cause of seizures in the developing world. NCC is increasingly diagnosed in developed countries due to growing population of immigrants from endemic areas and travelers to these areas. *T. solium* has two host life patterns. Ingestion of contaminated pork meet results in human taeniasis. Ingestion of *T. solium* eggs by fecal-oral transmition results in cyst formation in various organs named cysticercosis, including the brain (NCC). No data is available regarding the prevalence of NCC diagnosed in Israel and the prevalence of T. *solium* carriers. Herein, we present the results of the first nation wide survey performed in Israel.

Methods: A nation–wide survey was conducted in which all major hospitals in Israel were contacted. All cases of cysticercosis were documented. We collected data regarding the place of acquisition, symptomatology of the patients, imaging results and other diagnostic procedures used.

Results: During the years 1994-2004, 10 cases on NCC were diagnosed in Israel..

5 of the cases were found among Israeli travelers to endemic countries. 4 cases were diagnosed in immigrants from endemic areas. One case was found in an Israeli Arab citizen with no travel history.

Presenting symptoms were seizures in 50% of them and headaches. Diagnosis was frequently made by brain imaging, but serology was also used, and in two cases a brain biopsy was made. Stool test was positive in one case.

Discussion: NCC has become a greater concern also in developed countries. Israel is facing similar problem due to a large population of travelers, immigrants and foreign workers coming from endemic areas.

Since acquiring of cycsticercosis is via contaminated food with *T. solium* eggs, rather then eating contaminated pork meat; vegetarians are at same risk as the rest of the population. People at close contact with *T. solium* carriers are also at high risk. Although we would expect infection rate to be as high as other fecal-oral transmitted diseases, our data show much lower numbers.

In one case NCC was diagnosed in an Israeli citizen with no history of travel, which might indicates that the disease is endemic also in Israel.

In conclusion, high suspicion should rise when an individual first presents with neurological manifestations after visiting an endemic area. Physicians' awareness of the unique imaging findings could prevent unnecessary invasive procedures.

Gene expression changes during *Leishmania donovani* differentiation

Lahav, T.[2], Saxena, A.[1], Holland, N.[2], Aggarwal, G.[1], Anupama, A.[1], Huang, Y.[1], Myler, P.J,[1] and Zilberstein, D.[2]

[1]Seattle Biomedical Research Institute and Department of Biology, [2]Technion – Israel Institute of Technology, Haifa

L. donovani promastigotes transform into axenic amastigotes under conditions of low pH and high temperature. To investigate the molecular basis of this differentiation process, RNA was isolated at various time-points after exposure to the differentiation signal, and used to probe microarrays containing PCR-amplified DNA from a random-amplified genomic library of *L. major* Friedlin. Multiple repetitions of microarray hybridizations, as well as control labeling experiments, were carried out to quantify natural and experimental variations. Statistical analysis of the data after normalization revealed that several hundred genes were up or down regulated during differentiation. There were 121 promastigote-specific genes (including PFR, glycosomal GAPDH, β-tubulin, several protein kinases, several peptidases, several T-complex components, eIF-5a, and eIF-3 subunit 7) and 131 amastigote-specific genes (including SHERP, amastin, ABC transporters, pteridine transporters, protein kinases, DNA and RNA helicases, amongst others). In addition, 171 genes were up-regulated and 113 down-regulated transiently during the differentiation process, including a number of protein kinases and phosphatases, methyltransferases, heat shock proteins, peptidases, and helicases. In addition, the relative abundance of transcripts containing telomeric sequences appears to be regulated during the differentiation process.

Characterization of a DNA methylated binding protein in the protozoan parasite *Entamoeba histolytica*

Lavi, Tal and Serge Ankri

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

DNA methylation and histone modifications are epigenetic modifications involved in the regulation of genomic functions such as differential control of gene expression. We identified by immunodetection the presence of 5-methylated cytosine (m5C) in the parasite *Entamoeba histolytica*. The synthesis of m5C is catalyzed by a cytosine-5 DNA methyltransferase (Ehmeth) that belongs to the Dnmt2 proteins family. Methyl-CpG binding proteins (MBDs) mediate histone deacetylase-dependent transcriptional silencing at methylated CpG islands. Based on BLAST search, we were unable to identify homolog of MBDs in *E. histolytica* genome. However, we showed by Southwestern blot analysis that a 32 kDa protein present in *E. histolytica* nuclear lysate bound with higher affinity methylated DNA encoding reverse transcriptase of LINE (RT LINE) compared to non-methylated DNA. Using affinity chromatography with methylated RT LINE DNA as ligand, the 32 kDa protein was isolated and identified by mass spectrometry as a protein of unknown function. To characterize the biochemical properties of this protein called EHMBD, the clone was expressed in *E. coli* as GST-tagged protein. The recombinant protein showed higher affinity for methylated RT LINE. EHMBD represents a new family of DNA binding protein that may contribute to the regulation of gene expression in the parasite.

Plasmodium falciparum invasion of the red blood cells

Lustigman, Sara

Laboratory of Molecular Parasitology, Lindsley F. Kimball Research Institute, New York Blood Center, 310 East 67 th Street, New York, NY 10021, USA

A growing body of evidence indicates that the risks of acquiring malaria and of developing severe complications are determined by host genetic factors and the genotype of the infecting parasite strain. It is believed that the relationship between polymorphisms of red blood cell (RBC) surface antigens -- the RBC receptors for the merozoites -- and/or variations in *Plasmodium falciparum* ligands that participate in parasite invasion can determine the outcome of infection in malaria endemic areas. Our research is focus on the phenomenon of invasion of the *P. falciparum* merozoite into the RBC in order to understand how the interplay of host RBC polymorphism and parasite invasion ligand polymorphism affects the route of entry of the parasite. Although RBC specificity is dependent on ligand-receptor interactions it appears that these are not static in *P. falciparum*, partly to provide greater flexibility to the parasite. Merozoites can invade through several pathways, using different red cell receptors including GPA, GPB, GPC and Band 3 and others that are not as yet identified. Field isolates may show even greater variability in receptor utilization than do the laboratory isolates in which these pathways have been defined.

Initial studies that we have done in the Amazonian region of Brazil have shown that the presence of the GPB S allele on the RBCs was associated with incidence of *P. falciparum* while its absence was highly prevalent in the non-infected individuals who have been living in the same regions and were exposed to infections. It therefore appears that in these regions GPB is used more frequently by the parasites for invasion.

In our field studies we found 4 invasion pathways in 14 *P. falciparum* field isolates from Mato Grosso, Brazil, one of which was dependent only on sialic acid and firstly described in field isolates. Moreover, our studies have established that in comparison to those reported in the 3D7, Dd2, 7G8 and HB3 laboratory isolates these isolates have a limited repertoire of EBL-140, EBL-181 and the 4 PfRH ligand variants. More importantly they exhibited new PfRH1, PfRH2b and PfRH4 ligand variants. The impact of the distinct distribution of the defined polymorphic PfRH ligand variants on invasion was studied using principal component analysis. We found clear association between one particular variant in each of PfRH1, PfRH2a and PfRH2a with the NsTrCr pathway of invasion. None of the PfRH4 variants clustered with any particular invasion pathway. We hypothesize that the variability in sequence and expression of different members of these protein families may give these parasite populations a significant advantage in coping with polymorphisms in host red cell surface molecules as well as in evading immune responses. In order to overcome variability in host cells, they might make use of a variant domain within a ligand to bind to different receptors. The study of ligand polymorphism is important as it may provide clues to the level of selective pressure exerted on these proteins, specifically the functional RBC binding domains. Since merozoite antigens that function in the invasion process are the major focus of vaccine studies, information on sequence polymorphism, in particular in *P. falciparum* isolates from different endemic regions,

is highly relevant to vaccine development.

Onchocerciasis among Ethiopian immigrants in Israel – The Hadassah experience

Maayan S, Anteby I, Abramson N, Amer R, Amit Y, Bergshtein-Kronhaus T, Cohen E, Greenberg Z, Jonas F, , Marva E, Strauss U, BenEzra D. Enk CD

Departments of Dermatology, Clinical microbiology and Ophthalmology Hadassah University Hospital , Maccabi Health Services , District Health Office, MOH, Jerusalem

Background: Onchocerciasis results from infestation by the nematode Onchocerca volvulus, and is characterized clinically by itching, skin lesions and eye manifestations. Since 1992, approximately 9,000 immigrants have arrived in Israel from the Kuwara province of northwest Ethiopia where the prevalence of onchocerciasis is particularly high.
Objectives: To determine whether onchocerciasis is the cause of cutaneous and ocular symptoms among recent immigrants from the Kuwara province in Ethiopia.
Methods: We examined 1,200 recent immigrants from the Kuwara province residing at the Mevasseret Zion immigration center near Jerusalem. Among them, patients with cutaneous signs suggestive of onchocerciasis underwent a skin-snip biopsy and a thorough eye examination.
Results: In the detailed skin examination performed in 83 patients, the most common skin finding was chronic papular onchodermatitis, found in more than 46 patients (55%); depigmentation and atrophy was found in 13 (15%) and 12 (14%), respectively. In 40 patients (48%), living microfilaria were detected in their skin snips. Of the 65 patients who underwent a through eye examination, 45 patients (66%) had ocular complaints. Corneal abnormalities were found in 55 of the 130 eyes (42%), active anterior segment intraocular inflammation and live microfilariae were found in 4 eyes (3%) and lens changes in 16 eyes (1%). Eleven eyes (9%) showed retinal or choroidal changes.
Conclusions: Skin and eye manifestations associated with onchocerciasis are prevalent among symptomatic Ethiopians who immigrated to Israel from the Kuwara province.

***Anaplasma centrale* major surface protein 2 variants are generated by recombination of pseudogenes into a single expression site of the msp2 gene**

Molad T.,[1] Michaeli S.,[2] Mazuz M.,[1] Fleiderovich L.,[1] Leibovitz B.,[1] Krigel Y.,[1] Fish L.,[1] and Shkap V. [1]

[1]Kimron Veterinary Institute, [2]Bar-Ilan University

Anaplasma, a member of the Anaplasmataceae family, is an intra-erythrocytic pathogen, containing a circular genome. Two Anaplasma spp. infect cattle: *A. marginale* and *A. centrale.* Major surface protein 2 (MSP2) of *A. centrale,* used for vaccination of cattle, is encoded by a polymorphic multigene family. Antigenic variation of Anaplasma MSP2 occurs during acute and persistent infection and allows the rickettsiae to evade the pre-existing immune response. MSP2 of *A. centrale* was found to be composed of conserved amino- and carboxy-terminal regions flanking a central hypervariable region, and to be expressed from the operon of four open reading frames (ORFs) with msp2 at the 3' terminus. The operon appeared to be the only expression site of the full-length msp2 transcripts. The sequences of the three upstream orfs are conserved, while the polymorphism was found within the msp2-coding region in the hypervariable region. Seven msp2 pseudogenes were identified in the *A. centrale* genome. The pseudogene copies of msp2 in the genome are truncated, they contain a central hypervariable region flanked by short portions of the 5' and 3' conserved regions. *A. centrale* MSP2 variants are generated by two mechanisms: recombination of the whole pseudogene into the single msp2 expression site, or recombination of small segments of pseudogenes into the expression site by segmental gene conversion.

Traveling with children

Neumann, Karl

Clinical Associate Professor of Pediatrics, Weill Medical College of Cornell University, New York; Clinical Associate Attending Pediatrician, New York Presbyterian Hospital–Cornell Medical Center, N.Y., USA

Travel provides children with knowledge and experiences that enriches their education, builds their self confidence, promotes family cohesiveness, and creates memories for tomorrow. Preparing infants and young children for overseas travel requires expertise in pediatrics and travel medicine: knowledge of when infants can safely travel by air; awareness of disease patterns in countries that families will visit; altering routine childhood immunizations and administering travel-related vaccines; familiarity with the pediatric doses and contraindications of antimalarials and other medications – and what parents are to do when children refuse to take medications or vomit them; and educating parents about safety issues, proper food and water selections, altitude sickness, exposure to extreme heat and cold, the hazards of traffic, and what to do when children become ill in remote areas far from medical care.

Air travel and health

Potasman, I. and B. Brenner

Bnai Zion & Rambam Medical Centers, Haifa

The volume of air travel grows by 50% each decade. The maximum length of flights is also expected to grow by 50% in a few years with the advent of mega-planes. Consequently, more people will spend more time on long-haul flights. Passengers now make 1,500 billion miles per year. Much has been done and studied about pre-travel preparations, and treatment of post-travel diseases. This presentation will focus on problems associated with long-haul flights. Two distinct entities have been thoroughly studied in association with flights: infectious diseases and deep-vein thrombosis (economy class syndrome). Among the former, airborne transmitted diseases such as tuberculosis, influenza and SARS are notorious. 18% of all cases of sudden death among travelers arriving at Heathrow airport were caused by PE. The rate of PE was 150-fold higher in those who flew > 5000 Km, as compared to those who flew <5000 Km. The causes and possible treatments of DVT will be discussed.
But additional problems may arise on flights: the cramping position in the economy class may aggravate orthopedic problems; the quality of air and hypoxia within the passengers' cabin may be deleterious to COPD patients; what about emergencies aboard the aircrafts? Are we prepared? It has been estimated that for every ten million passengers, 225 acute in-flight incidents and one death will occur. Are we equipped for the elderly /pediatric /handicapped traveler? Neuropsychiatric problems may also exacerbate in the aircraft, and jet lag may occur soon after. The time is ripe to further the study of in-flight health conditions.

Malaria – Prevention and treatment

Schwartz, Eli

The Center for Geographic Medicine and Department of Medicine C, The Chaim Sheba Medical Center, Tel Hashomer

Malaria prevention in travelers to endemic areas is still based mainly on chemoprophylaxis. Malaria chemoprophylaxis is usually given for all malaria species. However, a distinction should be drawn between *Plasmodium falciparum* malaria prophylaxis and prophylaxis of relapsing malaria (*P. vivax* and *P. ovale*). Whereas the emergence of drug resistant strains, adverse side effects to drugs and the cost of the drugs complicate *P.falciparum* prophylaxis, *P.vivax* prophylaxis is almost non-existent.

The treatment of *P.falciparum* malaria is aimed at the blood stages of the parasite only. Due to rapid emergence of drug resistance, there is a constant need for new drugs. The old drug "quinine" is still very effective, but new drugs are also available. Atovaqone-proguanil (malarone) is an oral treatment suitable for uncomplicated malaria cases. Artemisinine-based compounds are now the drugs of choice in Africa. In Western countries the artemether-lumefantrine combination (Riamet®, Coartem®) has been registered. In contrast, treatment of *P. vivax* malaria is still based on chloroquine, as chloroquine resistance is rare. However, the liver-stage drug primaquine is needed for the completion of treatment. Under-dosing with primaquine was probably the reason for recurrent relapses of *P.vivax* malaria and therefore doubling the previously prescribed dose of primaquine has recently been recommended.

Two alternative routs for spliced leader RNA biogenesis

Shaked, Hadassa, Gil Arvatz, Itai Tkacz, Moshe Biton, Michal Mandelboim, Xue-hai Liang and Shulamit Michaeli

Faculty of Life Sciences, Bar-Ilan University, Ramat-Gan

In trypanosomes all mRNA carry at the 5' end a common spliced leader sequence which is donated to the mRNA from a small RNA, the SL RNA. In recent years, we demonstrated that SL RNA like snRNAs bind Sm proteins. There is currently a debate where in the cell Sm assembly on SL RNA takes place. The study from the Tschudi and Ullu labs suggested that unique cap-4 modification, and Sm assembly take place co-transcriptionally in the nucleus. However, studies from the Campbell lab suggested that SL RNA is exported to the cytoplasm via XPO1, where it undergoes the last step of cap-4 modification and Sm assembly before re-entry to the nucleus. We observed that during Sm depletion by RNAi the majority of the SL RNA is found in cytoplasmic speckles and also suggested that Sm assembly may take place in the cytoplasm. In this study. we demonstrate that during depletion the defective SL RNA lacking the +4 cap modification first accumulates in the nucleus, suggesting that Sm assembly takes place in the nucleus. Only after massive accumulation the SL RNA migrates to the cytoplasm but not via XPO1. In the cytoplasm the RNA is found in a unique particle the SL RNP-C that represents a novel post-transcriptional processing route to dispose of SL RNA when its normal biogenesis is blocked. We have purified the SL RNP-C to homogeneity and identified four of its binding proteins. The biological role of these proteins will be discussed. We will also present data suggesting that SL RNA assembly with Sm proteins takes place in a special compartment within the nucleus. Our data suggest that SL RNA biogenesis can go into two alternative pathways, the normal "productive" pathway which takes place in the nucleus and an alterantive "destructive" pathway in the form of SL RNP-C which is a dead-end pathway and removes the SL RNA from the nucleus to the cytoplasm where it is eliminated. The decision on which pathway SL RNA takes, depends on whether SL RNA can assemble Sm protein and undergo part of the 3' trimming.

Post travel morbidity in Israeli travelers

Steinlauf, S., I. Heiman and E. Schwartz

The Center for Geographic Medicine and Department of Medicine C, The Chaim Sheba Medical Center, Tel Hashomer

Background: There is a limited amount of information available about the morbidity of patients returning from the tropical regions. This is especially true for travelers returning to Israel. The existing information focuses mainly on hospitalized patients with febrile diseases. We have evaluated all causes of post-travel visits to our medical center.

Methods: The medical histories, diagnoses and destinations of patients (inpatient and outpatient), seen from January 1999–December 2004, with a history of recent travel were recorded. The demographics and destinations of healthy travelers seen in our pre-travel clinic during the same period were recorded. These healthy pre-travelers were used as controls.

Results: Of the 817 patients seen during the 5-year period, 497 (61%) were males and 320 (39%) were females. The most common problems encountered were: gastrointestinal (mainly chronic diarrhea) (34%), febrile diseases (33%), skin disorders (22%) and fatigue (18%). Patients with fever accounted for most of the admissions. Among these, malaria was the most common disease and was diagnosed in 89 patients (33% of all febrile diseases). Seventy-nine (29%) patients had unidentified febrile disease, 70 (26%) were diagnosed with dengue, and 17 (6%) had typhoid fever. Forty- eight percent of the patients had been to Asia, 23% to Africa and 32% to the Americas. Of our healthy traveler population, 59% traveled to Asia, 20% to Africa and 20% to the Americas. Thus, travel to Africa carried the highest risk of being hospitalized (OR, 1.9; 95% CI, 1.2–3; p=0.01). The most common disease in patients returning from Africa was malaria (41%). The principal health problem originating in Asia was chronic diarrhea (42%) and dengue fever (21%), and from Latin America skin disorders (40%) with cutaneous leishmaniasis being the most common (45 patients). Males were more likely to acquire malaria (OR, 2.1; 95% CI 1.1-4.1; p=0.02) and leishmaniasis (OR, 3.4; 95% CI, 1-11.9; p=0.05) than females.

Conclusion: Malaria, unidentified febrile diseases and dengue fever were the most common febrile diseases. Diseases were destination related; travel to Africa was associated with a higher rate of hospitalization. Malaria and cutaneous leishmaniasis had a substantially male predominance, probably due to risk-taking behavior.

ELISA and western blot analysis of serologic reactivity to *Leishmania tropica* in the Rock Hyrax (*Procavia capensis*)

Talmi-Frank Dalit[1], Charles L. Jaffe[2], Alon Warburg[2], Roni King[3], Milena Svobodova[4], Abed Nasereddin[2,5], Ahmed Amro[5] and Gad Baneth[1]

[1]School of Veterinary Medicine, Hebrew University, Rehovot; [2]Department of Parasitology, Hebrew University-Hadassah Medical School, Jerusalem; [3]Nature and Parks Authority, Israel ; [4]Department of Parasitology, Charles University, Prague, Czech Republic; [5]Center for Health Research, Al Quds University, Abu Deis, Palestinian Authority

Introduction: Outbreaks of cutaneous leishmaniasis (CL) caused by *Leishmania tropica* have been recently described in Israel and the Palestinian Authority. Infection was associated with the presence of rock hyraxes (*Procavia capensis*) in locations where human disease occurred, and infected sand flies were trapped in rock boulders where hyraxes live. In addition, *L. tropica* DNA was detected in the tissues of several hyraxes by PCR and a parasite strain was isolated from a hyrax in the Korazim region in northern Israel. Serological assays for reactivity of hyrax sera with *L. tropica* antigen were developed in order to investigate the possible role of the hyrax in the epidemiology of *L. tropica* CL, and to further evaluate its potential for serving as a reservoir host for this infection.

Materials and Methods: Sera were collected from hyraxes trapped in Maale Edomim during 2005. *L. tropica* promastigote antigen (LRC -1239) was used for ELISA and western blot assays. Sera from hyraxes raised in a zoo in a non-endemic area served as negative controls while sera from experimentally-infected hyraxes and from two naturally-infected PCR-positive humans served as positive controls. For w. blot, *L. tropica* proteins were separated on a 10 % SDS-PAGE, transferred onto a nitrocellulose membrane and reacted with sera.

Results: Thirty hyrax sera were tested by ELISA and compared to sera from a hyrax experimentally-infected by intramuscular inoculation, to another hyrax infected experimentally by sand fly bite, and to 6 hyraxes born in the Tel Aviv Zoological Garden