Tuesday, December 11, 2007

Session 5: General parasitology

A *var* specific nuclear factor is involved in transcriptional activation of virulence genes in the malaria parasite *Plasmodium falciparum*

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The major antigenic ligand of *P. falciparum* infected red blood cells is protein encoded by the multi-copy *var* gene family called PfEMP1. Each individual parasite expresses a single *var* gene at a time, maintaining the remaining ~60 *var* genes found in its genome in a transcriptionally silent state. In the present study, *P. falciparum* parasites were transfected with concatameric episomes carrying *var* promoters that had been disabled in silencing and were thus constitutively active and not recognized by the mechanism that controls mutually exclusive expression. By using increasing levels of drug pressure, it was possible to select parasite populations that carried similarly increasing numbers of active, episomal *var* promoters. We show that forcing parasites to express increasing numbers of transgenic *var* promoters resulted in gradual down regulation of transcription of all endogenous *var* genes, ultimately resulting in silencing of the entire family. The observed down regulation was *var* specific and did not affect parasite growth or the expression of housekeeping genes. Transient transfection assays using constructs containing heterologous promoters further confirmed that the down regulation was specific to *var* promoters. Interestingly, when drug pressure was removed and the episomes were shed by the parasites, they did not return to their previous *var* gene expression pattern, but rather displayed random *var* gene activation, indicating that the epigenetic memory marks that had previously regulated *var* gene expression had been completely erased. The data are consistent with the existence of a limiting, titratable *var* specific nuclear factor that is required for *var* gene activation.

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

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DNA methylation and histone modifications are epigenetic modifications involved in the regulation of genomic functions such as differential control of gene expression. Classical methyl-CpG binding proteins (MBDs) mediate histone deacetylase-dependent transcriptional silencing at methylated CpG islands. Previously, we have reported EhMLBP, an *Entamoeba histolytica* nuclear protein that serves as a sensor of methylated repetitive DNA in the parasite. To better understand the role of EhMLBP we have

knocked-down its expression using antisense vector. We observed a significant interference in the growth rate and cytopathic activity of the trophozoites transfected with the antisense vector. EhMLBP is unique to *Entamoeba* species and it does not share homology with human proteins. This feature makes EhMLBP a perfect target for new drugs against this parasite. A screen for inhibitor molecules using the phage-display method has been performed. Several peptides that compete with the binding of EhMLBP to methylated LINE retrotransposon (RT-LINE) are actually studied. In addition, we have identified with the Two-Hybrid screen analysis two proteins P1 and P2 that interact with EhMLBP. P1 shows some homology with a yeast protein involves in gene silencing. Like EhMLBP, P1 appears to bind with high affinity to methylated RT-LINE. P2 has no homology to proteins of known structure and it does not bind to methylated RT-LINE. We are currently examining the cellular localization of P1 and P2, their binding domains to EhMLBP and their roles in the parasite.

Polymorphism of the single copy BV80 gene in *Babesia bovis* isolates from distinct geographical regions

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Effective control of bovine babesiosis is achieved by vaccination with attenuated live parasites of *Babesia bovis.* Disease outbreaks sporadically occur among vaccinated cattle, however tools to discriminate between virulent field and attenuated vaccine strains of the parasite are not yet available. Conventional PCR, RT-PCR and sequence analysis of the single copy BV80 gene were applied to characterize and differentiate the vaccine isolates derived from cattle blood or culture, and field virulent isolates from Israel and Uzbekistan. Two different sets of primers amplified multiple amplicons ranging between 450-800 bp in all field or vaccine isolates, except for a single fragment obtained with the DNA from a calf-derived vaccine MH strain. Sequence analyses of the Israeli and Uzbek strains confirmed presence of mixed parasite subpopulations and extensive polymorphism in attenuated and virulent isolates. It appears that the BV80 is not a suitable marker for discrimination between virulent and attenuated parasites. The selection pressure and mechanism(s) that lead to preferential growth of the attenuated parasite population which comprise the vaccines strain are not yet elucidated.

Predator-released kairomones repels oviposition by the mosquito *Culiseta longiareolata*

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Previous work showed that *Culiseta longiareolata* (Diptera: Culicidae), a potential vector of West Nile Virus, are strongly repelled by one or more kairomones released by the aquatic predator, *Notonecta maculata* (Hemiptera: Notonectidae) when ovipositing. Isolating the kairomone can potentially provide an environmentally friendly method of mosquito population reduction. Using a behavioral assay, we found that the mosquito is repelled without having to touch the water indicating that at least one component of the kairomone is highly volatile. Volatile compounds were extracted from *Notonecta*-conditioned water using Solid Phase Microextraction (SPME). Two hydrocarbons, heneicosane ($C_{21}H_{44}$) and tricosane ($C_{23}H_{48}$), which were found in *Notonecta*-conditioned water, but not in control water, were tested as potential oviposition repellents in outdoor artificial pool experiments using synthetic compounds of these hydrocarbons. Our preliminary results indicate that both compounds cause oviposition repellency and that the combined effect is additive.

Mosquito oviposition and the aging effect

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We studied the effect of physiological age on the oviposition behavior of *Aedes aegypti* following two successive gonotrophic cycles using a choice experiment under laboratory conditions. Blood-engorged mosquitoes (n=10) were introduced into individual cages that contained four oviposition sites (choices) with different salinities: Distilled water (DW), 0.33% NaCl (T1), 0.67% NaCl (T2) and 1% NaCl (T3). The oviposition substrate (seed germination paper) was changed daily and the number of oviposited eggs (the response variable) was recorded. The initial oviposition activity ceased on the tenth day of the experiment and two days later a second blood-meal was offered to the original cohort of mosquitoes; only five became visibly blood-engorged. The oviposition activity was again followed for 12 additional days. During the first oviposition period (days 1-10 of the experiment), the mosquitoes oviposited predominately in the DW and T1 treatments and completely avoided the T3 treatment ($p<0.001$). In contrast, the oviposition pattern during the second gonotrophic cycle (days 12-24 of the experiment)

was less polarized, suggesting that the physiologically older mosquito may be less selective. The implications of these data are discussed in the context of lethal ovitrap strategies for the control of urban container breeding mosquitoes.

Session 6: Vector-borne diseases

An update on ehrlichiosis and other rickettsial diseases in Israel – a veterinary perspective

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Rickettsial diseases have become increasingly important in companion animal medicine in Israel and worldwide in recent years. More rickettsial organisms, some of which are zoonotic, have emerged to infect companion and wild animals and to be involved in disease processes.

Ehrlichia canis: Canine monocytic ehrlichiosis is the most common infectious disease affecting dogs in Israel. Recent studies investigating treatment protocols and prognostic indicators for the disease have shown that dogs recover from acute canine monocytic ehrlichiosis after 16 days of doxycycline treatment (10 mg/kg of body weight every 24 h) (Harrus et al., 2004). Moreover, pronounced pancytopenia (WBC<4 x 10^9/L; HCT<25%; PLT<50 x 10^9/L) was found as a risk factor for mortality. Severe leucopenia (WBC<0.93x10^3/µL), severe anemia (PCV<11.5%), prolonged activated partial thromboplastin time (APTT >18.25 seconds) and hypokalemia (K<3.65mmol/L) were each found to predict mortality with a probability of 100% (Shipov et al., In publication). These prognostic indicators may be useful aids for the clinician when treatment and prognosis are being considered.

Anaplasma phagocytophilum: *Anaplasma phagocytophilum*, infects and causes a clinical disease in humans and a wide variety of animals. Antibodies reacting with the organism have previously been demonstrated in Israel, both in humans, canines and the golden jackal (*Canis aureus syriacus*). Recently, *A. phagocytophilum* DNA was demonstrated for the first time in Israel in *Hyalomma marginatum, Rhipicephalus turanicus* and *Boophilus kohlsi* ticks collected from roe deer (*Capreolus capreolus*) on the nature reserve in Mount Carmel (Keysary et al 2007). This finding warrants the attention of physicians and veterinarians to this animal and zoonotic pathogen.

***Rickettsia conorii*:** The agent of Israeli spotted fever was recently associated with an acute, febrile illness in 3 male Yorkshire terriers from Sicily (Solano-Gallego et al., 2006). The prevalence of IgG-antibodies reactive with an Israeli strain of *Rickettsia conorii* was examined in humans and dogs from 2 rural villages in Israel. Sixty-nine of 85 (81%) canine sera and 14 of 136 (10%) of human sera had anti-*R. conorii* antibodies. The study indicated that exposure to spotted fever group rickettsiae was highly prevalent among dogs compared to humans in the two villages examined, probably reflecting a greater exposure rate of canines to the tick vector. The results suggest that canine

serology is a sensitive indicator for the presence and magnitude of human exposure to *R. conorii* (Harrus et al., 2007).

Rickettsia felis: *Rickettsia felis*, an emerging bacterial pathogen, causing a flea-borne spotted fever in humans was recently detected in 7.6% of 79 cat flea (*Ctenocephalides felis*) pools from Israel. This was the first detection of this flea-transmitted rickettsia within its vector in Israel and the Middle East (Bauer et al., 2006). Although no clinical case has been reported in human beings in Israel to date, these findings suggest that this infection is prevalent in Israel.

Equine trypanosomiasis in Israel – A new diagnosis

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Trypanosoma evansi is a haemoflagallate of the subgenus Trypanozoon and is the pathogen causing the animal disease called "Surra" in Africa and Asia, and "Mal de Calderas" in South America. It affects a number of species mainly horses, camels and cattle. *Trypanosoma evansi* is mechanically transmitted by blood-sucking flies mainly of the genus *Tabanus* and is not considered a zoonosis. Surra is mainly a wasting disease and is characterized by chronic weight loss, icterus, edema, anemia and neurological signs. An acute onset with high mortality is also possible. In November 2006 the first case in Israel of equine trypanosomiasis was diagnosed in the Veterinary Teaching Hospital of the Hebrew University of Jerusalem. The mare in question was referred to the hospital with a history of chronic weight loss despite good appetite, and deterioration over the last few days with fever and general weakness. The mare resided in the southern Dead Sea area in a small tourist resort where a camel was diagnosed with trypanosomiasis a few months earlier. On presentation at the hospital, the mare had normal vital signs, was emaciated and had neurological signs consistent with a central vestibular lesion. CBC and blood smears showed non-regenerative anemia, elevated WBC with marked lymphocytosis. Several trypanosome parasites were found on Giemsa-stained blood smears, buffy-coat smears and in a CSF cytology sample collected from the lumbo-sacral region. After ten days, her condition deteriorated and she was euthanized. Necropsy and histopathology findings were consistent with trypanosomiasis, however not specific. Further diagnostic tests were performed later. A card agglutination test (CATT) for detecting specific antibodies against *T. evansi* was applied on serum collected from the mare and was found to be positive. Additional sero-positive animals (a horse, donkeys and camels) were found in the same farm where the mare resided. No parasites were detected in blood smears from these animals. PCR, with *T. evansi* specific primers, was applied on tissue-DNA extracted at necropsy from the mare. An approximately 400bp single band was found in the brain stem, spinal cord and bone marrow. Sequencing of the band demonstrated the closest similarity to *T. evansi*. A large scale survey of the prevalence of *T. evansi* in the equine population in Israel went on its way last month. Diagnosis is based on serology, blood smears and PCR. The first region that was sampled was the Arava and Dead Sea area. This region was chosen as the first one since the

positive animals were found in this region and the fact that positive animals were found in neighboring Jordan. Initial results showed that out of 140 sampled animals (134 horses, 4 donkeys and 2 camels) 8 horses were serologically positive. Only one of these horses showed clinical signs that could be attributed to Surra. No parasites were found so far in any of the examined blood smears. Other areas of the country will be sampled in the coming months.

Control of ectoparasites and vector-borne diseases

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In view of the strong focus on hygiene and preventive care in small animal practice we have to ask ourselves as veterinarians whether parasites, and especially ectoparasites, still play any significant role in our patients. The answer to this question is an unequivocal yes. Ectoparasites, which include ticks, fleas, sandflies and mosquitoes are as prevalent as ever in dogs and therefore require our attention. Especially the tick is a very important ectoparasite and vector of human and animal disease agents. Ticks together with mosquitoes are the two large groups of blood-feeders that play an important role in veterinary medicine as well as public health. Ticks however transmit a greater variety of infectious organisms than any other group of blood-sucking arthropods. Today in small animal clinics worldwide a large variety of ectoparasiticides are available specifically developed for the use on companion animals. Besides the insecticidal and acaricidal properties of these compounds and their proven effectiveness, the view today has shifted especially in canine medicine, from ectoparasite control towards prevention of canine vector borne-diseases (CVBD). Pathogens transmitted by acarids and insects remain the major thread to companion animals and especially dogs may be exposed to a variety of pathogens while it has been confirmed that e.g. ticks may harbour more than one pathogen. In endemic areas, it is common that different ectoparasites feed on their mammalian hosts; a very common combination is e.g. ticks and sandflies. Thus prevention of tick attachment or blood-feeding of flying insects is today in focus of parasitologists, internists and clinicians. In terms of tick infestation, one has to understand the complexity of tick biology and esp. the complex mechanisms of tick feeding, to differentiate the use of various acaricides and their therapeutic and more important, prophylactic properties. Prevention of tick bite is mainly through prevention of attachment. It may be possible to prevent transmission of pathogens by killing ticks soon after attachment, because most tick transmitted diseases require some hours of attachment prior to transmission. However, the goal is to prevent attachment in the first place and thus any pathogen transmission if possible. The principle of prevention has become firmly established in veterinary medicine. Thus the basic rule prophylaxis over therapy, which applies in the case of farm animals, should also be applied in small animal practice, whenever suitable products are available. To achieve this, acaricides with repellent properties, such as the synthetic pyrethroid permethrin are ideal compounds to reach this goal. In the combination with the fast acting insecticide imidacloprid, both

actives are effective against the three major ectoparasites ticks, sandflies and fleas. The ectoparasiticide containing the combination of imidacloprid and permethrin has in well controlled studies demonstrated the effectiveness in blocking the transmission of e.g. *Borrelia burgdorferi* and *Anaplasma phagocytophilum* from naturally infected ticks, or *Leishmania infantum* from sandflies to dogs. Reducing the interaction between the ectoparasite and the dog as the mammalian host, means reducing the risk of transmission of pathogens that may lead to CVBDs, and this is key to achieve the well-being and health of dogs.

Session 7: Travel medicine

A profile of Israeli travelers to endemic countries: a national survey

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Introduction: During each of the last 3 years, there were 3.6/3.7 million departures of Israelis traveling abroad as reported by the Israeli Central Bureau of Statistics[1], yet little is known about the destinations or purposes of their travel or whether or not they had accessed health services before traveling.
Methods: A telephone survey that was conducted in November 2007 by Geocartography investigated this subject. The sample size consisted of 2,000 households, which is a representative sample of the adult (>18 years old) Jewish population, (sampling error +/- 2.19%, at 95% CI).
Results: Based on this survey, it was found that from 2004-07 about 160,000 Israelis traveled each year, to developing countries including areas endemic for malaria. 61% traveled to Asia, 32% traveled to Central and South America and 10% traveled to Africa. The purpose of these visits in the majority of cases (74%) was tourism. The pattern of travel by age shows that travelers to Central & South America were younger, mean age 41.3 yrs, while the travelers to Africa were the older, mean age 49.1 yrs. Only half of the travelers to Africa, Asia and the Americas accessed any health services before traveling. Of those who did access pre-travel services, 39% went through Kupat Holim or Primary Care Physician (PCP) while less than 10% accessed care through a traveler clinic and 8% went through the regional Ministry of Health (MOH) offices. The great majority of those who accessed care through a PCP or Kupat Holim were not referred on to a travel clinic or a regional MOH office. This finding is in contradiction to most PCPs' claims that in most cases they do refer such travelers to a travel clinic or a Regional MOH office. Half of those who accessed health services were not given any recommendations by the physician for medications to prevent malaria, and 12% of those travelers who were given medication did not take it. For the most part, those who did not access any pre-travel care were in the age range of 35-54.
Conclusion: There is a large number of Israelis who travel to endemic areas where pre-travel medical services are needed. In spite of this, approximately 50% do not seek

medical advice. In addition, the older travelers who are often at higher risk due to co-morbidities are the least likely to seek medical advice.

The profile of Israeli travelers – Travel clinic perspective

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Introduction: Traveling to endemic countries has become very popular for Israelis of all age groups. Despite the large number of travelers, their profiles have not been well described. In this study we aimed to look at the demography, destination and purpose of the trips in large cohort of Israeli travelers.

Methods: Data of travelers presenting at the travel clinic at the Chaim Sheba medical center from Jan 1999 through December 2006 were analyzed. Demographic data were described in entirety while travel destinations, and trip durations were recorded from randomly selected patients.

Results: During this period, 36,253 traveler's records were analyzed. The average age was 30.5±13.2 and 54.0% were males. Pediatric travelers (<18 years) made up 5.5%, and elderly population (>60 years old) made up 4.3% of the total travelers population. Southeast Asia was the most common destination visited by the travelers (55%), followed by Africa (25%) and Latin America (18%). The median trip duration was 30 days, the 25^{th} percentile was 14 days and the 75^{th} percentile was 90 days. The overwhelming majority (87%) of travel to tropical countries was for pleasure, 6% went for business, and 7% went as representatives for governmental organizations. There were several changes which occurred during the study period. There was a significant increase in the rate of females traveling, increasing from 41.7% in 1999 to 48.5% in 2006. There was a steady increase in the number of travelers seen by our clinic, with the exception of 2003, when the number declined coinciding with the SARS epidemic. Interestingly, the Tsunami, which occurred in Asia at the end of 2004, did not affect the number of travelers seen in our clinic. The distribution of travel destinations varied significantly during the study period. Of note is the sharp decline in travel to Africa following the terrorist attack in Mombassa, Kenya in 2002.

Conclusion: There is a steady increase in the number of people who attend our travel clinic, which may reflect either an increased number of travelers or increased awareness of the need for pre-travel consultation. Although young adults make up the majority of travelers, all ages including pediatric and geriatric travelers are venturing to endemic areas and these groups especially may need particular attention in relation to vaccines and prophylactic drugs.

Personal protection measures for prevention of insect bites - efficacy and limitations

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By employing Personal Protection Measures (PPMs) everybody, in theory, is able to protect himself against arthropod induced hazards. PPMs include adopting preventive behavior; the use of space insecticide/repellent combinations; application of topical repellents and the use of permethrin treated materials. Repellents for space treatment as well as for topical application are either synthetic chemicals, or plant derived chemicals, especially oil distillates. Various formulations and methods of application are available that also differ in the type and amount of active ingredients. It is important to stress that a "natural ingredient" is not necessarily equally effective or a safer product. There is no scientific evidence to show that eating garlic, vitamins, onions, or any other food will improve a person's repellency to biting arthropods. The attraction of each individual to biting arthropods is based on a complex interaction of many chemical and visual signals. Certain foods in certain individuals may affect their individual attractiveness to biting arthropods, for better or for worse. Proper and overlapping use of PPMs can provide close to 100% protection. In practice this is only partially achieved. Many factors contribute to the limited efficacy of PPMs: difficulty in active implementation on a daily basis; reluctance to use products because of suspicious safety and/or unpleasant odors and sensation; improper use of products and a shorter than indicated protection time. The current PPMs available to the public in Israel, their benefits and limitations, as well as future methods of improving their efficacy are discussed.

Air-evacuations of Israeli travelers – analysis of 7 years

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Introduction: According to the Israeli Central Bureau of Statistics, there are about 3.5 million departures of Israelis traveling abroad annually. Little is known however about their medical problems while abroad, and their need for medical evacuation has never been assessed. This is the first study aimed to summarize the reasons for medical evacuation of Israeli travelers from all parts of the world including Western countries, Eastern Europe, and developing countries.
Methods: All air evacuations that occurred during the years 2000-2006 by one major Israeli company (Air-Med), were analyzed. All air evacuations that necessitated medical personnel assistance are included. Evacuations within Israel or evacuations for Israelis who were flown abroad for medical treatment and needed medical escort on the way back were excluded.
Results: During the 7 years period, 430 evacuations occurred. The majority of evacuations were from Western countries and account for 55%, developing countries account for 27%, and Eastern Europe 18%. Males account for 66% of all evacuations from all destinations. The mean age of patients evacuated from the developing countries was lower in comparison to those evacuated from the West (42 vs. 63 years old). Altogether, the reasons for evacuations were medical in 61.5% and trauma in 38.5% of all cases. In the Western and Eastern Europe countries, the majority of evacuations were due to medical reasons (65%), and trauma accounts for only 35%, while in the developing countries trauma and medical conditions accounts for 50% each. The most common cause for medical evacuation was cardiovascular disease in all regions (48% in developing countries, 56% in western countries, and 68% in Eastern Europe). Interestingly, infectious diseases accounted for only 7% of the medical evacuation in the developing countries while in the Western countries it accounted for 18%.
Conclusion: In Israel, there are no official statistics about the destinations of Israeli travelers, however a national survey indicated that traveling to developing countries account for about 5% of all travel and yet the rate of evacuations from these regions accounts for 27% of cases. Despite poor medical conditions in developing countries, only a minority were evacuated due to infectious diseases or vaccine preventable disease.

Ministry of Health guidelines for international travel – update 2006-7

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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 and periodically update a pamphlet for travellers including general as well as more specific recommendations regarding health precautions and preventive measures. Recent updates include hepatitis A pre exposure prophylaxis, issues regarding meningococcal revaccination, MMR vaccination for travelers, plaquenil instead of chloroquine for malaria prophylaxis and use of Korean Japanese encephalitis vaccine instead of Biken Japanese encephalitis vaccine.

Session 8: Travel & Tropical medicine

Management and treatment of malaria in the non-immune returning traveler: a 2007 update

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25-60% of the identifiable causes of fever in returning travelers is caused by the malaria parasite depending on the geographic location of the clinics and the destinations of travelers. Every year there are around 30,000 cases of malaria in travelers and case fatality rates are 1-4% in most industrialized countries. Non-immunes with *Plasmodium falciparum* malaria can rapidly deteriorate so early treatment is critical to a favorable outcome. We will review a logical approach to the febrile patient, initial recognition and management dilemmas, and the choice and use of currently available agents.

Risk and prevention of tick-borne encephalitis in travelers

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Tick-born encephalitis (TBE) is endemic in several countries of Europe and Asia. Over the past decades TBE, Lyme Borreliosis, and other tick-borne diseases have become a growing public health problem in Europe and other endemic regions. It can be stated that global warming causes some of these dramatic changes. Also in travel medicine we are facing a growing problem since an increasing trend of Central and Eastern European travel destinations has been observed. However, travelers going to Europe do not feel like travelers exposed to possibly travel-associated infections since problem of awareness

among tourists as well as doctors is rather scanty. However, with increased intercontinental travel to areas of high endemicity tourists run incremental risk of acquiring TBE during their holiday. On the basis of excellent epidemiological data available in Austria, a considerable risk of TBE disease can be estimated for an unvaccinated tourist staying for 4 weeks in a highly endemic touristy province of Austria. Active immunization is the most effective tool of TBE prevention, showing cross-protection against various TBE virus subtypes. Though not formally evaluated, it seems reasonable to recommend TBE vaccination to travelers going to high-risk TBE regions. Moreover, growing awareness outside endemic areas may provide the basis for monitoring of TBE infections throughout Europe enabling us to calculate a more accurate risk of TBE infection in unprotected travelers to high risk areas.

Acute schistosomiasis outbreak following a safari trip to Tanzania

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Background: Acute schistosomiasis (Katayama Syndrome) outbreaks were previously described among adventure travel groups. The signs and symptoms of acute schistosomiasis in travelers differ from the clinical presentation of schistosomiasis in the local population. Little is known regarding the economic burden of acute schistosomiasis outbreaks. We describe the demographics, clinical course and economic impact of an acute schistosomiasis outbreak in a group of travelers acquired in a single exposure during a safari trip to Tanzania.

Patients and Methods: Two groups that spent one day in a tented lodge in Tanzania had a single brief exposure to an adjacent fresh water pool. All group members were examined and followed after an index case was suspected. Clinical symptoms, laboratory and serologic findings were documented. A questionnaire regarding the loss in work days, school days, leisure activities and the amount of medical encounters was answered by all group members.

Results: During their stay, 27 of 34 travelers in the groups were exposed to the swimming pool. Among them 22(81%) were infected and 19 became symptomatic. Comparing the infected vs. non-infected group, the exposure time was longer in the infected group (44±35 minutes versus 12±11 minutes respectively, p=0.06). In the infected group, 19/22 (86%) developed symptoms. Among the symptomatic group, fever was present in 68% of patients, cough in 78%, rash and diarrhea in 37% and 10% reported angioedema. Cercarial dermatitis was described in only 3 patients (15%). The total number of work and school days missed by the infected group was 152.5 days (mean of 7 days per infected patient), 258 medical encounters were reported (mean of 11 encounters per patient) and 329 leisure activities were missed (mean 15 activities per

patient). Four patients were hospitalized with a total of 29 hospitalization days. All patients completely recovered and resumed normal activities.
Conclusion: A single short exposure of a group of travelers to an infected pool led to a high infection rate. Infected travelers suffered work and leisure losses and required extensive medical investment in evaluation and treatment with the economic implications attached. Education to avoid exposure to fresh water sources in endemic areas remains the hallmark of schistosomiasis prevention in travelers to endemic areas.

Complications of yellow fever vaccination: A case report and review of the literature

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Yellow fever (YF) is endemic in Sub Saharan Africa and South America. In the last decade several cases of severe neurological diseases (YF-AND) and multiorgan system disease (YF-AVD) have been described. Although older age was found to be a risk factor for YF-AVD, no contraindications were set for this population. We describe an 82 years old male who received a YF vaccine prior to planed travel to Brazil. He presented to our hospital seven days after vaccination, with a history of two days of fever up to 39.9, chills, headache and malaise. Notably, on same day of the YF vaccination he also received typhoid and tetanus diphtheria vaccination. Laboratory results on admission were remarkable for disturbed liver function tests including elevated bilirubin and a thrombocytopenia. During hospitalization the patient suffered from extreme weakness and shortness of breath, but improved with symptomatic therapy. Blood viral cultures were positive for the 17D YF- vaccine strain. Over the last years a more detailed risk assessment for YF- vaccination was published. A reported rate ratio of 13 for YF-AVD was described in adults above 70 years of age. As the age of travelers continuously increases and travelers in the very old age group are seen with increasing numbers, a careful risk benefit assessment should be done before immunizing this adult population.

Session 9: Leishmania (Epidemiology)

Leishmania and HIV in the Mediterranean region

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Visceral leishmaniasis (VL) can be life threatening for immuno-suppressed HIV patients. Both HIV and VL infections suppress the immune system, rendering the patient more susceptible to opportunistic infections by pathogenic microorganisms and parasites.

There are several indications that the morbidity of leishmaniasis is increasing in different countries and is related to concomitant HIV infections. These co-infections are considered to be emerging conditions and have been reported from 35 countries worldwide but most of the cases notified to the WHO are from four countries in south–western Europe; France, Italy, Portugal and Spain. Although most of the Mediterranean VL was seen in infants, more than 85% of the co-infected patients were 20–40 years-old, mainly young male adults. In most countries, HIV occurs mainly among drug abusers, prostitutes and their clients, or homosexuals. Natural transmission of VL is via the bite of the sand fly but drug abusers who are often also infected with HIV are at high risk of infection through needle sharing. There is also the possibility of being infected by blood transfusion. Reactivation of asymptomatic or healed leishmaniasis is common in AIDS patients and HIV infection can alter the natural history of leishmaniasis, impede rapid diagnosis, and decrease the efficacy of antiparasitic treatment. On the other hand, leishmaniasis can facilitate the transmission of HIV and accelerate progression from asymptomatic HIV infection to AIDS. AIDS patients subsequently become vulnerable to opportunistic diseases such as tuberculosis, toxoplasmosis and candidiasis. The "gold standard" for the diagnosis of leishmaniasis is direct microscopic observation of amastigotes in the splenic and/or bone marrow aspirations after Giemsa staining. Molecular techniques are more reliable, however, expensive and time consuming. Serodiagnosis by ELISA, IFAT, DAT and FAST is relatively easy to perform and in the case of the last two tests do not require sophisticated equipment. However, each one alone is not always reliable for immuno-compromised patients, as the antibody production of these patients is impaired, therefore, the use of two or more serological tests or moleculear methods is recommended. In Turkey, a 49 year old heterosexual male living on the Mediterranean coast was found as co-infected with both HIV and Leishmania. The presence of antibodies against *Leishmania infantum* in the patient sera was confirmed by IFAT, DAT, FAST and rk39 dipstick assay. The present case shows that in endemic areas of Mediterranean region, VL should be considered a possible opportunistic infection in HIV patients with fever, hepatosplenomegaly and anemia. Adult patients especially, those suffering from VL and are unresponsive to treatment, relapsing or developing opportunistic diseases, should be checked for HIV.

Leishmaniasis in the Jordanian army

(Abstract not received)

Epidemiology of visceral leishmaniasis in The Palestinian Authority

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Leishmania infantum causes human visceral leishmaniasis (VL) in The Palestinian Authority where it is primarily a disease of infants and young children. A few studies describe the situation. Currently, two regions are affected, the Jenin and Hebron Districts in the northern and southern West Bank, respectively. The former comprised 50 (39%) of the 127 cases recorded between1989-1998. Sero-positivity measured by an ELISA in 1,100 school children and 148 domestic dogs from the Jenin District was 7.5% and 6.8%, respectively; and five strains from dogs and one from a child were shown to be *L. infantum* by ITS-1 and kDNA PCRs and their excreted factor (EF) serotype (B2). Enzyme analysis showed that two of the strains from dogs belonged to the universal zymodeme MON-1 and the human one represented the new zymodeme, MON-281. The Palestinian Ministry of Health reported 53 cases aged between 1-12 years old from the Hebron District during 1994-2004. An additional five cases were reported in 2007 that were confirmed by the serological rk39 test, and two strains were isolated from bone marrow aspirates. They were also EF sub-serotype B2 and belonged to zymodeme MON-1. DNA analysis using ITS1 sequencing and RFLP revealed that they were identical to the reference strain *L. infantum* IPT1. ELISA Sero-positivity was 7.2% in Beit Ola from where 38% of the cases in the Hebron District came. Sand flies were collected, revealing several putative vectors but no promastigotes were seen on their dissection. VL has been present in these active foci for a long time and further investigation is required to identify the animal reservoir and vector.

Cutaneous Leishmaniasis among IDF soldier

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Background: Cutaneous Leishmaniasis (CL) is hyper endemic in different regions in Israel. Military service in Israel is mandatory at the age of 18 so the majority of the Army population, related to Leishmaniasis, is very young. CL is a mandatory reportable disease in the IDF and in the civilian ward. In 2003-4 an outbreak of CL caused by Leishmania major occurred in southern Israel.
Objective: To describe the epidemiology of CL in the last 10 years among the soldiers population.
Methods: Physicians all over the army bases are constructed regarding this disease in the primary course and more intensively for physicians in the endemic areas. Every case of CL has been investigated by the physician and the epidemiology section of the Army Public Health Branch as like as the Ministry of Health for the civilian sector. The diagnosis of reported CL was based on the clinical presentation, relevant epidemiologic data, and microscopic visualization of the parasite in most cases. During the period of the outbreak, a multidisciplinary investigation was initiated, which included active surveillance, dissemination of questionnaires, physical examination and laboratory testing of suspected lesions. Annual CL incidence rates in the civilian population were derived from the Israel Ministry of Health publications.
Results: Consistently, the average rate of CL among soldiers was higher than the civilian sector. The majority of the Leishmania isolates in the IDF have been *L. major*, while in the civilian sector there is a recent rise in cases of *L. tropica*. These differences are likely

caused by frequent exposure opportunities to the disease among the soldiers population, differences in using medical services between soldiers and civilians and perhaps the active surveillance that is frequent only in the Army. Related to the Army cases, the vast majority were infected in the south of Israel. Country of birth, military occupation and insect repellent use were not correlated with CL infection.
Conclusion: There is a high incidence of CL among soldiers who were infected in the south of Israel. This data emphasizes the need for multidisciplinary efforts, civilian and military for the development and the implementation of effective measures for preventing CL. Regional and international cooperation are needed. A task force composed from the Ministry of Health, Ministry of Environment, Medical Corps, Israel Nature and Park authority, was established in 2005 to build together a comprehensive prevention program.

The epidemiology of cutaneous leishmaniasis in Israel

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Cutaneous leishmaniasis is a zoonotic disease, endemic and modifiable in Israel. The vectors are sandflies of the genus *Phlebotomus* and the hosts are mainly field rodents. The infective agents are Leishmania parasites. *Ph. papatasi* is the recognized vector of *L. major*, while *Ph. sergenti* is considered to be the vector of *L. tropica*. Three peaks in incidence, 1967-1969, 1980-1982 and 2004-2005 were observed. These peaks reflect environmental changes caused by the introduction of non-immune people, mainly Jews, into the area of endemic foci, enhanced urbanization by expansion of settlements bordering this area, agricultural and industrial projects and also possibly the effect of global warming. In recent years, most reported cases were from the Tiberias sub-district, the Southern district (the Negev and the Arava) and the Jerusalem district (Maale Adumim). Based on available laboratory data, in Maale Adumim the main species seen was *L. tropica*, in the Negev and the Arava, *L. major*, and in Tiberias and the northern Galilee, *L. tropica*. In the Northern District and Judea there were also sporadic cases in which *L. infantum* was implicated. Cooperation of the Ministries of Health and Environmental Protection in combating the vectors and the reservoirs in animal hosts must be continued.

Ancient DNA-based pathogen research with emphasis on visceral leishmaniasis in Nubia and Egypt

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Research in ancient pathogen DNA has progressed and results on tuberculosis, leprosy or plague are now accepted and confirmed. However, very little has been done in ancient parasite DNA. We shall discuss our recent work on leishmaniasis in ancient Nubia and Egypt. The former is often thought to be an important early focus of this disease and nowadays it is endemic in several parts of the country. Human remains from two cemeteries at the medieval site of Kulubnarti in Sudanese Nubia have been the focus of intensive research since 1979. They represent ancient agricultural communities of the early (AD 550-750) and the late (AD 750-1450) Christian periods. Over 400 naturally mummified individuals were recovered in an exceptional state of preservation due to the dry environment. The few cases of leishmaniasis so far detected can be examined from the perspective of ecology, diet and associated diseases in this community. For example, a coprolite collection from this population, expected to reveal a *plethora* of intestinal parasites, was in fact very poor. Instead good evidence was provided to reveal that garlic, cloves and other vermicides/vermifuges in the diet may explain this paucity. Tissue from the pelvic basin also failed to reveal *Schistosoma haematobium* parasites. However 30% individuals were found positive for tuberculosis and in these cases it was possible to show that all of the organisms were *Mycobacterium tuberculosis* and not *M. bovis*. Lately, evidence for leishmaniasis has been found in 9 individuals among the 70 bone marrow samples examined from the Nubian mummies. The presence of the vector of visceral leishmaniasis, *Phlebotomus orientalis*, is positively associated with the presence of *Acacia seyal*, and traces of *Acacia* sp. have been recorded in the coprolites (L.S. Cummings 1989). Using primers for minicircle kinetoplast DNA, we are now able to provide evidence that leishmaniasis was not only present in Nubia in this early Christian period, but also that the organism infected ancient Egyptians. It has been postulated that the disease arrived in Egypt due to close trading contacts to Nubia during the Middle Kingdom. We have analyzed 91 bone samples from ancient Egyptian mummies and skeletons. The material derived from the Pre- to Early Dynastic site of Abydos (n=7; 350-2800 BC), a Middle Kingdom tomb in Thebes West (n=42; 2050-1650 BC) and different tomb complexes in Thebes West, which were built and used between the Middle and New Kingdom until the Late Period (n=42; c. 2050–500 BC). All samples were tested for Leishmania DNA and further characterized by direct sequencing, resulting in 4 positive *Leishmania donovani* complex cases.

Session 10: Leishmania (Veterinary)

New concepts on canine leishmaniasis

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Canine leishmaniasis is a major zoonotic disease endemic in more than 70 countries in the world. It is present in regions of southern Europe, Africa, Asia, South and Central America and has recently emerged in the USA. Canine leishmaniasis is also an important concern in non-endemic countries where imported disease constitutes a veterinary and public health problem. Dogs are the main reservoir for human visceral leishmaniasis and the disease is usually fatal if not treated in people and dogs. Phlebotomine sand flies are the vectors of *Leishmania infantum*, the causative agent of canine leishmaniasis in the Old World and for its New World synonym *Leishmania chagasi*. Seroprevalence rates found in studies carried out in the Mediterranean basin range between 10 and 37% of the dogs in endemic foci. Surveys employing the polymerase chain reaction (PCR) method for the detection of leishmanial DNA in canine tissues, or combining serology and DNA detection, have revealed even higher infection rates approaching 70% in some foci. It has been estimated based on seroprevalence studies from Italy, Spain, France and Portugal that 2.5 million dogs in these countries are infected. The number of infected dogs in South America is also estimated in millions with high infection rates in some areas of Brazil and Venezuela. Population studies in *Leishmania*-endemic areas have shown that a proportion of the canine population develops a symptomatic disease, another fraction has persistent asymptomatic infection, while yet another fraction is resistant to the infection or intermittently resolves it without developing clinical signs. Immune-mediated mechanisms are responsible for much of the pathological findings in canine leishmaniasis. Circulating immune complexes and antinuclear antibodies have been detected in animals with canine leishmaniasis. Glomerulonephritis associated with the deposition of immune complexes in the kidneys is a hallmark of the disease. Renal pathology is present, even if not manifested clinically, in the majority of dogs infected with this *L. infantum*. Susceptibility or resistance to canine leishmaniasis is influenced by genetics. A study on the polymorphism of the canine NRAMP1 gene has implied that susceptible dogs have mutations in this gene. In addition, a dog major histocompatibility complex (MHC) class II allele has been linked to the risk of being infected in an endemic area in Brazil. Canine leishmaniasis is a good example of a disease in which infection does not equal clinical illness due to the high prevalence of asymptomatic infection. This makes canine leishmaniasis a diagnostic challenge for the veterinary practitioner, clinical pathologist and public health official in endemic countries as well as non-endemic regions where imported infection is a concern.

Control of canine leishmaniasis

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Infection in dogs with the protozoan parasite *Leishmania infantum* is widespread in the Mediterranean basin. Furthermore, *Leishmania* infections in both dogs and humans is distributed worldwide, with first reports recently been published also from the US. *Leishmania* is a vector-borne pathogen and thus prevention of the sandfly bites is a prerequisite to reduce the risk of canine as well as human leishmaniasis in areas where the disease is endemic. In the absence of any vaccination or chemoprophylaxis, the regular application of insecticides throughout the sandfly season should be the standard of good veterinary practice. By doing so, the individual dog within an endemic region as well as the one traveling to an endemic region may be protected. In addition, transmission from already infected dogs to the sandfly population and spreading of the disease is blocked. No guidelines on how to conduct studies to demonstrate efficacy against sandflies are currently in place. Thus the already known repellent effect of an imidacloprid/ permethrin combination from on-animal laboratory studies was tested under field conditions. The efficacy of the combination of imidacloprid 10% and permethrin 50% (Imi/Per) in a spot-on formulation was evaluated in the field as a control measure to prevent canine leishmaniasis (canL) in an endemic area of southern Italy. In this investigation, 845 asymptomatic dogs were enrolled into a field study in two kennels. All dogs were initially tested for canL and 631 dogs were negative in a serological and a parasitological examination (lymph-node cytology and PCR on skin samples) while 208 (24.6%) were found positive. Negative dogs were allocated to one of three groups: Group A - treated with Imi/Per once a month; Group B - treated every two weeks; and Group C – as untreated control. Within the groups, the dogs were evenly distributed ($P<0.05$) for individual characteristics (sex, age, weight, coat length). All the dogs were examined serologically and parasitologically for canL prior to the start of the sand fly season, at the end of the sand fly season and again prior to the following sand fly season. The sand fly population density was monitored in both kennels throughout the season from April to November. In the kennel of Bari *Leishmania* infection, inferred by positivity in at least one of the three tests performed at the interim or final follow-up, was found in one dog from Group A and in 9 from Group C. No positive dogs were detected in Group B, thus giving a final protection efficacy of 88.9% in Group A and 100% in Group B. In the second kennel (Ginosa) *Leishmania* infection was identified in one dog from Group A, one from Group B and 11 from Group C (protection efficacy of 90.36% in Group A and 90.73% in Group B). The incidence density rates of infection in both Groups A and B at each kennel were significantly lower than that registered in Group C (KB $p<0.05$ and KG $p<0.01$). Sand flies first appeared in June and disappeared by mid-October in both kennels, and *Phlebotomus perniciosus* was the most common sand fly species identified (58.6%). The results clearly demonstrated that a combination of Imidacloprid/Permethrin,

due to its repellent activity against sandflies, was effective to prevent canine leishmaniasis.

Human and Canine Leishmaniasis in Turkey: Updates

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In Turkey, human visceral leishmaniasis (HVL) and canine leishmaniasis (CanL), caused by *Leishmania* (L.) *infantum* MON-1, are endemic along the Aegean and Mediterranean coasts and occur sporadically in other regions. Anthroponotic cutaneous leishmaniasis (ACL) is still highly endemic in the south and southeast regions. According to the Turkish Ministry of Health official records, within a period of 7 years (2000-2006) an overall of 203 human visceral leishmaniasis (HVL) and 15.908 cutaneous leishmaniasis cases were reported in Turkey and most of them occurred in the South Anatolia, Mediterranean and Ege Regions. The number of epidemiological studies on leishmaniasis, including vector identification, has risen in recent years in Turkey. Dogs are the reservoir of this protozoan parasite, *L. infantum*, which is transmitted among canines and to humans by phlebotomine sand flies (Desjeux, 2004) and the direct role of infected dogs in the epidemiology of human VL is controversial. Several diagnostic tests such as indirect fluorescence antibody test (IFAT), ELISA, rK39 dipstick are available to detect anti-*Leishmania* antibodies in human and canine sera. These tests combine high sensitivity and specificity. Additionally, molecular techniques and parasitological examination in the clinical samples have also been performing in some labs in Turkey. Previous studies of sand fly fauna in Turkey revealed 19 *Phlebotomus* species (or subspecies recently raised to species level) belonging to the *Phlebotomus, Adlerius, Larroussius and Paraphlebotomus* subgenera. It is also known that there are five *Sergentomyia* species belonging to the subgenus *Sergentomyia*. Nine of these species are proven or probable vectors of the parasites causing human leishmaniases in the Old World. Although CanL was first detected in the early 1950's, the knowledge about the epidemiology of this disease was limited in Turkey. First CanL cases were reported from Bursa (1951) and Istanbul (1954) cities. The first epidemiological study was showed 1.6% seroprevalence rate using IFAT among 1150 dogs from Aegean and Mediterranean regions in 1981. There was no detailed study till 1993. After 1993, epidemiological studies were started to perform in different provinces where the human visceral leishmaniasis (VL) cases were reported previously. The clinical status of the dogs was also carefully evaluated. So far, the epidemiological studies were carried out in 22 different provinces. During the field work, the collaborations with Ministry of Health and veterinarians working in government were established and veterinarians were trained theoretically and practically. The prevalence ratios have been changed between 1.45%

and 27.5% and the overall prevalence of CanL was found to be as 11.32% in Turkey. Seven dogs were treated successfully using “long term treatment protocol”. These parasitologically proven dogs were treated with a combination of allopurinol and sodium stibogluconate for nine months. Currently, 33 *Leishmania* strains were isolated from dogs and 11 of them were identified as *Leishmania infantum* MON-1 (same in human) and 3 of them was found to be *L. infantum* MON-98. The molecular studies for identification of the strains were also carried out and the results showed that CanL isolates had three different patterns and one of them is identical with all human VL isolates. Our laboratories has been acting as unofficial reference laboratory for the diagnosis and/or confirmation of human and canine leishmaniasis in Turkey. Because of absence the national leishmaniasis control program except treatment in Turkey and CanL has more importance in the medical and veterinary fields, starting of CanL awareness and control program will be advised to the government. For this reason, an informative poster has been prepared for veterinarians and dog owners about canine leishmaniasis. In addition, a 20 minutes of footage has been prepared. This video movie includes information about visceral, cutaneous and canine leishmaniasis and its vectors. There is a need to get together and compare all the information in order to a better understanding of the epidemiology of the disease and the necessities for the future efforts.

Session 11: Leishmania (Clinical aspects and advanced methods for diagnosis)

The US Military Infectious Disease Research Program (MIDRP) Leishmaniasis Research Program Update: The Diagnostics Portfolio

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Following the US lead military invasion of Iraq in March 2003, the US military began seeing cases of cutaneous leishmaniasis (CL) in the fall of 2003. The vast majority of these cases were typical CL caused by zoonotic *L. major*. The sudden increase in cases led to the re-establishment of a an active research and development program in the US military focused on improved vector control measures (Program Area U) and improved diagnostics and therapeutic agents for case management (Program Area P). The current status of the Diagnostics portfolio include: 1) a point of care, non-microscopic dipstick diagnostic device, 2) improved field friendly PCR platforms, 3) An interferon gamma release assay to detect asymptomatic infections, and 4) status of a Leishmania skin test antigen. An overall view of the worldwide status of diagnostic testing directed at CL and where the US military program can contribute to the known capability gap will be reviewed.

Advances in molecular diagnosis of leishmaniasis

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Classical diagnostic techniques for the leishmaniases are based on clinical signs and symptoms, parasite identification by microscopic examination and culture, and in some cases serological assays. While still useful these techniques are primarily hampered by poor specificity and sensitivity, as well as additional disease and parasite associated problems such as poor leishmanial growth in culture, contamination, low antibody titers, serological cross-reactions with other diseases, etc. Modern molecular methods are providing clinicians and researchers with better tools for diagnosis. Immune chromatography using recombinant polypeptide rK39 provides a rapid, specific and sensitive test for visceral leishmaniasis that can be used in the field, but is not useful in monitoring drug treatment. Antigen based tests for visceral leishmaniasis that allows indirect monitoring of patient parasite load during treatment is under development, but they must be validated. Polymerase chain reaction (PCR) is now widely used for leishmaniasis diagnosis, and numerous studies comparing PCR to the classical methods have been published. Many targets have been employed in PCR including nuclear and kinetoplast DNA, intergenic and protein coding DNA regions. Most techniques are genus specific however several, when combine with additional steps, can also be used for species identification; and molecular dipstick tests that combined combine PCR and oligochromatography are under development. Recently, new techniques such as quantitative nucleic acid sequence based amplification (QT-NASBA) and real time-PCR have been utilized. In addition to specificity and sensitivity, these assays allow one to monitor parasite load during drug treatment potentially permitting the rapid identification of parasite resistance. This talk will review the status of leishmaniasis diagnosis today and directions for future research.

Cutaneous leishmaniasis: A case control study on the effectiveness of treatment with a topical ointment (Leshcutan) in Israeli soldiers

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IDF Medical Corps

Background: Cutaneous leishmaniasis (CL) is caused by parasites of the genus *Leishmania* which are transmitted to humans from a rodent reservoir by sand flies. Two species of Cutaneous *Leishmania* are endemic to Israel, *Leishmania major* (most prevalent) and *Leishmania tropica,* both of which belong to the old-world group that causes only cutaneous manifestations. *Psammomys obesus* is the rodent reservoir for *L. Major*, and *Phlebotomus* sand flies are the vector. CL has been recognized as a serious public health problem in Israel and Israel Defence Forces (IDF) soldiers are conspired at high risk to the disease, due to personal, behavioural and environmental factors. There is

no single optimal treatment of CL. Most old-world CL lesions heal without treatment, but those on cosmetically or functionally important sites, and those with multiple or persistent lesions are best treated actively. Treatment usually consists of local or topical treatment, except in specific conditions which require systemic treatment. A 10 day topical treatment, twice daily, with an ointment containing 15% paromomycin and 12% methylbenzethonium chloride (Leshcutan), is widely used in Israel and in the IDF. In a small double blind control study done by El-ON J etal, 74% (29/39) of patients treated with leshcutan were cured, versus 27% (4/15) patients treated with placebo.

Objective: To study the effectiveness of Leshcutan used in CL treatment in Israeli soldiers and to determine the satisfaction of Israeli soldiers treated with Leshcutan for CL.

Methods: All computerized medical IDF records from 2004 were reviewed and all cases of diagnosed cutaneous leishmaniasis were identified. 100 cases of cutaneous leishmaniasis treated with Leshcutan and 100 controls of cutaneous leishmaniasis not treated with leshcutan were selected. Each case and control was interviewed by phone to complete a questionnaire. The questionnaire included socio-demographic details, time of presumed infection, clinical features, use of Leshcutan and satisfactory with treatment outcome.

Results: There was no difference comparing number of lesions between the cases and controls (4.3 and 3.6, t= 1, p=0.3). 23% (14/60) of cases had lesions on their face compared to 7.6% (3/38) in the control group (fishers exact test, p=0.059). The average subjective disturbance (on a scale of 1-10, 1 being no disturbance) was 7.9 in the cases, compared to 6 in the controls (t=2.85, p=0.05). Both groups found the appearance of the lesion the most bothersome feature. 21.7% of lesions healed completely (without a scar) in the cases group, compared to only 5.4% in the control group (fishers exact test, p=0.042). There was no difference in how long it took the lesion to heal (mean healing time in the cases group was 129 days, compared to 140 days in the control group, t= -0.399, p=0.691).

Conclusion: Leshcutan appears to positively affect the outcome of CL lesions, although it has no affect on the mean healing time. The differences in outcome might be due to clinical differences in the first place affecting the decision to give treatment, such as the location of lesions.

Treatment with Intra Lesional Pentostam for cutaneous leishmaniasis acquired in Israel

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Background: Israel is one of the endemic places for cutaneous leishmaniasis. *L. major* is the most prevalent species. Although L. Major causes only cutaneous disease, it can still be a disfiguring problem. The treatment options are very limited and there are not enough studies done to investigate new options. Intra Lesional Sodium Stibogluconate (SSG) is

one of the treatment options. In former studies the patients were treated by injections of SSG everyday or on alternate days over a 30-day period. In this study, we assessed our experience with Intra Lesional SSG.

Methods: A retrospective evaluation was conducted to examine cases referred to dermatology and the Center of Geographic Medicine. Diagnosis was made by smear or skin biopsy and in some cases PCR analysis for species specific diagnosis was added. Intra Lesional SSG was injected 0.5 cc per ulcer (50 mg).The treatment was repeated every 2-3 weeks.

Results: During the period 10/2004- 10/2006, 33 cases (26 males and 7 females) of *L. major* mostly in Israeli army soldiers with a total of 93 lesions, were treated with Intra Lesional SSG. Within three months of treatment initiation, complete healing of cutaneous ulcers occurred in 91% (30/33) of cases after a mean of 3 treatments (range 1-6). Side effects were mild, mostly pain during injection. Two patients developed mild cellulites in the site of injection.

Conclusion: Intra Lesional SSG treatment is safe, effective and well tolerated, with minimal side effects. The number of treatments required is relatively low and has the same cure rate as IV SSG or frequent intra-lesional treatments.