ABSTRACTS

Sunday, December 27, 2009

08:30 - 09:00 **Registration**

09.00 – 11.00 **Session 1: Mosquitoes and Sandfly-borne diseases**
Chairpersons: Alon Warburg & Hedva Pener

09.00 – 09.20 **Bin, Hanna, Leah Weiss, Sarah Schlezinger & Ella Mendelson. Arboviruses infecting humans in Israel: West Nile Virus, Sindbis Virus and Sandfly Viruses**

09.20 – 09.40 **Shalom, Uri, Gil Stav, Maayan Perkin, Hamza Habib Allah, Itay Lachmi, David Meir, Allon Bear, Yoav Lustigman, Abed Cyrati, Hezi Giladi & Tal Weinberg. The distribution and risk map of *Aedes albopictus* in Israel.**

09.40 – 10.00 **Baneth, Gad. Zoonotic Dirofilaria repens infection in Israel**

10.00 – 10.15 **Orshan, Laor & Lea Valinsky. Leishmania infections in pooled sand flies and blood fed females**

10.15 – 10.30 **Faiman, Roy, O. Kirstein, M. Torem, M. Freund, H. Guetta & A. Warburg. Incoming low and slow – Control of Phlebotomine sand flies with a large-scale vertical mesh barrier**

10.30 – 10.45 **Talmi-Frank, Dalit, Noa Kedem, Roni King, Charles L. Jaffe & Gad Baneth. An epidemiological study for the identification of Leishmania infection in wild canids in Israel**

10.45 – 11.00 **Discussion**

11.00 – 11.30 **Break**

Arboviruses infecting humans in Israel: West Nile Virus, Sindbis Virus and Sandfly Viruses

Bin, Hanna[1,2], Leah Weiss[1,2], Sarah Schlezinger[1,2] & Ella Mendelson[2,3]

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West Nile virus (WNV), Sindbis (SIN) and Sandfly viruses (SFV) are antigenically distinct arboviruses, belonging to different taxonomic groups (Flaviviruses, Alphaviruses, and Phleboviruses of the Bunyaviridae, respectively). All are endemic in Israel since the early 1940's. Vectors dictate viral seasonality which is late summer-autumn for WNV and SIN, and spring- summer for Sandflies. These viruses can also be imported by travelers or migratory birds. WNV causes periodic outbreaks and since 2000 has shown yearly variations in activity genotypes, virulence, and in temporal and spatial distribution. Three- country-wide outbreaks occurred in 2000, 2005 and 2007 with 439, 102 and 184 patients and 29, 3 and 6 deaths, respectively. The two main vectors are *Culex perexiguus* and *Culex pipiens*. Molecular analysis revealed one highly conserved genotype similar to the NY99 strain and several, divergent genotypes related to European or African strains. Repeated introductions of different genotypes can explain in part the epidemiology of WNV in Israel. SIN and WNV share similar zoonotic cycle, but differ in clinical symptoms and pathology. SIN causes fever, rash, swollen joints associated with prolonged poly-arthritic pain and occasionally encephalitis. Several SIN isolates were obtained from mosquitoes co-infected with WNV. Isolates were antigenically related to confined geographic regions in Israel, and ELISA assays based on a mixture of 5 isolates originating from 5 different geographic locations were developed. Primary SIN infections were found mostly in retrospective studies on WNV cases with probable recurrent infections since clinicians are still unaware of the virus and its symptoms. SFV are transmitted by sandflies (SF's) and 4 serotypes are endemic in Mediterranean countries, of which Toscana SFV is the most virulent, causing meningitis and meningoencephalitis. The other 3 SFV cause a Flu-like syndrome (Papatachi fever). Recent studies showed that vectors of all 4 viruses are endemic in Israel and seroprevalence detected 8% positive and 33% borderline cases with Tiberias, Eilot district, Michmoret and Jerusalem being endemic. In an outbreak which occurred in soldiers camping near Gaza strip (n=23) IFA IgM and IgG were conducted with 4 different antigens. Half were found to be recently infected. Cyprus and Sicily viruses were the main agents, but Toscana and Naples were also involved. 82% were exposed to at least one genotype. In Kibbutz Cabri, where SF's were found, 39% were exposed to at least 1 out of 4 viruses, mostly to Cyprus and Sicily viruses. Recent work on 4 WNV suspected patients showed for the first time, that a local SF Naples virus can be neurovirulent and cause meningitis or encephalitis. In conclusion, all 3 families of arboviruses are highly active in Israel but little is known about SIN and SFV biology pathology and epidemiology. Further studies are prompted.

The distribution and risk map of *Aedes albopictus* in Israel

Shalom, Uri [1], Gil Stav[2], Maayan Perkin[1], Hamza Habib Allah[1] Itay Lachmi[3] David Meir[3], Allon Bear[1], Yoav Lustigman[3], Abed Cyrati[1], Hezi Giladi[3] & Tal Weinberg[1]

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A study of the national distribution of the Asian tiger mosquito (*Aedes albopictus*) in Israel was motivated by the outbreak of Chikungunya in Italy in 2007 and the threat of local introduction of this disease. The study was conducted in 2008 by monitoring the presence of eggs in ovitraps placed in villages and towns that were believed to be infested with the mosquito. Each of the suspected places was also checked for the presence of larvae and adult of *A. albopictus*. Out of 260 municipalities, 44 were suspected and tested for presences of mosquitoes. 33 were found to be infested with *A. albopictus*. In 23 (of the 33) municipalities the presence was restricted to one location, whereas in the other 10 it was found in several locations. Unofficial reports from municipalities and pest management professionals and from our own observations indicate that in September 2009 the *A. albopictus* infestations were already present in 46 municipalities. A risk map for *Aedes albopictus* establishment in Israel was developed. We concluded that the overall risk for tiger mosquito establishment in Israel should be based on 3 main criteria: the ecological potential for the mosquito establishment in the future, the present distribution of *A. albopictus* in Israel and steps taken by municipalities to control the presence of this mosquito. We also concluded that, in the contrary to the European risk map, factors such as photoperiod, temperature and sea level were not relevant as criteria for *A. albopictus* risk map in Israel. We intend to check the feasibility of this model in 2010, and create a national risk map for Israel.

Zoonotic *Dirofilaria repens* infection in Israel

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Zoonotic filariasis caused by *Dirofilaria repens* has been reported from several regions in the world including Southern Europe, Africa, the Middle East and Southeast Asia. Dogs are the reservoir for this infection and people are accidental "dead end" hosts in which the life cycle is usually not completed. The mosquito vectors of *D. repens* vary in different geographic regions and include species belonging to the genera *Culex* and *Aedes*. *D. repens* in dogs and humans have been reported recently at an increasing rate from Central Europe as well as from Southern and Eastern European countries. Due to the increase in the number of human infections in Spain and Italy, it is considered an emerging zoonosis in these countries. Human dirofilariasis was first reported in Israel by Romano in 1976 and has since then been reported sporadically. The diagnosis of canine *D. repens* infection, although reported by Witenberg from Palestine in 1934, has not been reported thereafter until 1999 after which the frequency of the diagnosis has increased. *D. repens* is the only filarial worm known to be transmitted in Israel. Canine infection is often an incidental hematological finding, or accompanied by mild clinical signs including skin swelling, hyperpigmentation or prurutic subcutaneous granulomas containing adult worms. In humans, immature *D. repens* migrate in connective tissues and elicit an inflammatory response resulting in the formation of nodules around the worms. The manifestations of *D. repens* infection in people are associated with these nodules. *D. repens* nodules have been described from the lung, subcutaneous, epididymis, spermatic cord, lung, omentum, conjuctiva, and the breast. Twenty six cases of *D. repens* infection have been documented in dogs visiting veterinary clinics in Israel between 1998 and 2009. The majority of infected dogs were from the Galilee in northern Israel. In addition, infection was documented in 29/39 (74%) of dogs examined by the Knott's test and specific PCR from a kennel near Haifa. The epidemiology and vectors of *D. repens* in Israel should be investigated to learn more about its transmission and geographic spread. Detection of infection in dogs, correct treatment and appropriate prevention are important in order to control the spread of infection and its transmission to humans.

Leishmania infections in pooled sand flies and blood fed females

Orshan, Laor &d Lea Valinsky

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In Israel, cutaneous leishmaniasis (CL) is caused by *Leishmania major*, transmitted by *P. papatasi*, and *L. tropica* transmitted by *P. sergenti* and *P. arabicus*. In the last years the diseases emerged in new foci extending the geographical distribution. In 2005, this situation and the lack of effective control methods led the Israeli government to initiate an integrated research program, covering various aspects of surveillance and control. Participants are from the Ministry of Environmental Protection, the Armed Forces, the Nature and Parks Authority and the Ministry of Health. The goal is to define disease risks and to find solutions. Sand flies were collected from disease foci in the Galilee, Beit-Shean Valley, the Judean Desert, the Samaria Mountains and the Negev. The catch was sorted and counted. All males and samples of the females were identified. The majority of the females were pooled in groups of up to 20 for molecular detection of *Leishmania* DNA using PCR and RFLP of the ITS1 region. Engorged females were co-analyzed individually for *Leishmania* infection and the source of the blood meal. The estimated risk for disease transmission is based on sand fly densities, *Leishmania* infection rates and the rate of feeding on humans. Since 2005 approx. 60,000 unfed female sand flies were screened for *Leishmania* in pools and approx. 1,300 engorged females were analyzed individually. The results and preliminary analysis will be presented. The relevance for measuring changes and focusing attention to increased transmission risks will be discussed.

Incoming low and slow – Control of Phlebotomine sand flies with a large-scale vertical mesh barrier

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A 400 meter long insecticide-treated vertical net barrier was used to intercept foraging sand flies on the periphery of an agricultural village in the northern Jordan Valley (Kibbutz Sede Eliyahu). The surrounding Kibbutz fence was draped with a deltamethrin-impregnated PermaNet® (450 holes/inch^2). Sand flies were captured inside and outside the Kibbutz before and after draping of the fence, using CO_2-baited CDC traps or CDC light traps. Sand fly frequencies, as monitored around three houses along the barrier (Treatment group), exhibited an 84.9% decrease ($P<0.01$) after the barrier had been erected. Conversely, the neighboring control group of houses exhibited a 15.9% increase in sand fly abundance during the parallel time period. These results buttress previous findings from field tests on a small-scale (60 m) PermaNet® barrier in the urban setting of Ma'ale Adumim. Integrated vector control campaigns for reducing the burden of sand fly bites, should consider integrating outdoor vertical fine-mesh nets to reduce the numbers of sand flies arriving at inhabited areas. Coupled with indoor area repellants a significant reduction in bite burden and transmission of leishmaniasis in endemic regions is achievable.

An epidemiological study for the identification of *Leishmania* infection in wild canids in Israel

Talmi-Frank, Dalit[1], Noa Kedem[1], Roni King[2], Charles L. Jaffe[3], Gad Baneth[1]

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Introduction: The leishmaniases are endemic diseases in Israel and are of growing concern to public health in recent years. The three parasite species responsible for leishmaniasis in Israel are *Leishmania infantum*, *L. tropica*, and *L. major*. These *Leishmania* species have different sand fly vectors and reservoir hosts, therefore it is important to study the epidemiology and epizootiology of the disease in order to develop preventative measures. The domestic dog in known as the main peri-domestic reservoir for *L. infantum*, while different rodent species are reservoirs of *L. major*, and the rock hyrax is the main suspected reservoir for *L. tropica*. Little evidence is available on the possible role of wild canids in the epidemiology of leishmaniasis in Israel, and this information was based only on serological surveys. The aim of this study was to identify and characterize *Leishmania* infection in wild canids including jackals, foxes, and wolves in Israel using species specific real- time PCR and ELISA.

Materials and Methods: The study included a total of 113 tissue samples from 77 jackals, 25 foxes and 11 wolves, as well as 189 sera from 157 jackals (*Canis aureus*), 27 foxes (*Vulpes vulpes*) and 5 wolves (*Canis lupus*). Samples were collected at different locations in Israel including the Galilee, Golan Heights, Central Israel and the Arava by the Israel Nature and Parks Authority during 2000-2009. Different tissues including blood, spleen, lymph nodes, and skin of ears and snout were tested for the presence of *Leishmania* DNA using real-time PCR and High Resolution Melt (HRM) analysis. The PCR products were sequenced to verify the species identification. Antibodies reactive with *Leishmania* were evaluated using the ELISA.

Results: Leishmanial DNA was detected in 6 jackals (7.8%) and 2 foxes (8%), altogether in 8/113 (7%) of the animals tested. Five jackals and 2 foxes were positive for *L. tropica* DNA, and only 1 jackal was positive for *L. infantum*. Ear skin was infected in 6/67 (9%) of the animals tested and the spleen was positive in 4/26 (15.4%). Simultaneous infection of ear skin and spleen was found in 3/16 (19%). Species identification was confirmed using sequencing and BLAST analysis. Antibody titers higher then the cut-off level were found in 3.18% of the jackals and 3.7% of foxes collected in central and northern Israel. To our best knowledge, this is the first report of *L. tropica* infection in jackals and foxes.

Conclusions: These results imply that wild canids might play a role in the sylvatic cycle of *L. tropica* and *L. infantum* infections, and could act as sentinels for infections in endemic as well as new emerging foci.

Sunday, December 27, 2009

11.30 – 12.45 **Session 2: Tick-borne diseases I**

Chairpersons: Michael Samish & Michael Giladi

11.30 – 11.50	**Mumcuoglu, Kosta Y. Ixodid ticks in Israel: biology, epidemiology and vectorial capacity**
11.50 – 12.10	**Wilamovski, Amos & Heather Schnur. Soft ticks (Argasidae) in Israel**
12.10 – 12.30	**Assous, Mark V. Relapsing fever in Israel: new aspects of an old disease**
12.30-12.50	**Rudoler, Nir. Vector-borne diseases around Israel**
12.50 – 13.00	**Discussion**
13.00 – 14.00	**Lunch**

Ixodid ticks in Israel: biology, epidemiology and vectorial capacity

Mumcuoglu, Kosta Y.

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Ticks are temporary ectoparasites of animals and man. All feeding stages are blood-feeders. There are 42 tick species, which are endemic in Israel: 27 of them are hard ticks (Ixodidae). With the help of Haller's organ, a sensory organ capable in detecting CO2, odor and heat, ticks can localize their hosts. Ticks like Rhipicephalus and Ixodes wait on vegetation until a host animal comes close enough to allow them to attach themselves to the hair/cloth. Ticks like Hyalomma are quick runners and can find their host actively. Tick bites are characterized by a local erythema and the development of a granuloma, which can last for months. Incorrect removal of the ticks could lead to secondary infections and abscesses. Out of 7 Ixodes ticks known from Israel, *Ixodes redikorzevi* is known to cause tick toxicosis in human and *Ixodes gibbosus* tick paralysis in animals such as goats and sheep. Ticks together with mosquitoes are the most important vectors of pathogenic organisms. They can transmit the pathogens transovarially and transstadially but also by co-feeding. Ixodes species known to transmit Lyme diseases in North America and Eurasia are absent in Israel. The five known species of Rhipicephalus in Israel are known vectors of pathogenic bacteria such as *Rickettsia conorii, Ehrlichia canis, Ehrlichia chafeensis, Anaplasma phagocytophilum* and *Anaplasma marginale*, while *Hyalomma marginatum* and *Hyalomma detritum* are the vectors of *Anaplasma marginale*. Rhipicephalus species are also vectors of pathogenic protozoa such as *Babesia bovis, Babesia bigemina, Babesia canis, Babesia equi, Babesia cabali, Babesia ovis,* and eventually also of *Babesia gibsoni* and *Babesia felis* as well as of *Hepatozoon canis*. *H. marginatum* and *H. detritum* can transmit *B. equi, B. cabali* as well as *Theileria annulata* in horses and cattle. *H. marginatum* is a known vector of the Crimean Congo hemorrhagic fever (not present in Israel), which in the last years caused a lot of morbidity and mortality in Turkey.

Soft ticks (Argasidae) in Israel

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In 1967, Costa and Theodor recorded 6 species of *Ornithodoros* and five of *Argas*. Little additional information has been added since then concerning these species. The Argasidae species emerge from their hiding places when they detect a warm-bloodied host in the vicinity. The adults suck blood for 20 to 30 minutes while the nymphs feed for less time. The first larval stage attaches to the host for several days which explains the distribution method of the species by the host. The *Ornithodoros* species of Israel are mainly parasites of mammals. The best known one is *O. tholozani*, vector to man of *Borrelia persica*, the causative agent of relapsing fever (cave fever). Between 1980 and 2008, 245 cases of relapsing fever were recorded among Israeli civilians, an average of 8 cases per year. A greater number of cases were recorded among soldiers. The infection foci in 86% of the cases were caves; the rest were from ruins, archeological sites, animal dens and burrows. In all these sites, there must be a suitable microhabitat for the ticks to survive. *O. tholozani* is distributed all over Israel except for the southern Negev. In Israel, the mammalian host of the bacterium is unknown, but trans-ovarial transmission to the tick offspring has been recorded, thus *O. tholozani* is both the host and the vector of this bacterium. 92% of infected persons are walkers or youth entering the tick's living range without taking preventative measures. Only 7.5% were professionals working in caves, were aware of the risks and acted accordingly. *O. tholozani* may detect the host outside the cave or other biotopes up to a distance of 15 meters. In a survey carried out in the 1970s, 30-60% of all caves checked in the centre and north of Israel were found to be infested with *O. tholozani*. In a recent survey in 2004 in the same region, 46 % of caves were infested. The infestation of ticks with *Borrelia persica* ranged between 2% to 40%. The tick *O. coniceps* was known in Israel as a parasite of domestic chickens and hid in the upper parts of stone poultry houses which were common in the past. In 1953, it was found that this tick was also a vector of *Borrelia persica* causing disease in the area of Nablus. The changes in the building of poultry houses caused this tick to become very rare. In 1997, it was identified attacking a Jerusalem family in an upper story apartment, causing very severe reactions and one person was hospitalized. The infestation source were chickens being raised in a stone building in the yard of the apartment block. *O. lahorensis* has been recorded on goats from the north of Israel to Arad. The species of *Argas* are bird parasites. *A. reflexus* is found on doves and pigeons. A single case is known in which doves nesting on the roof of an Eilat house were a focus for this tick which attacked the house residents. In a 2005 survey, *A. reflexus* was found in one poultry house and a feral pigeon nest. *Argus latus* was first found in Israel in 3 Jerusalem sites in which the residents suffered severe bites. Large populations of pigeons found on the roofs of the houses and nearby are assumed to be the host of this tick. In all the sites, the ticks were hiding in cracks and crevices in the upper parts of the upper floors. *A. latus* was later found in other Jerusalem sites as well as Beer Sheba. *A. persicus* was found on chickens in chicken houses, but rarely on wild birds. It was common in Israel in the past, but the improvement in the construction of chicken houses and in their sanitary conditions has caused this parasite to become rare. A single population was detected recently from a chicken house in the Upper Galilee in 2005. *A. arboreus* is a parasite of the cattle egret, *Bubulcus ibis*.

West Nile Virus was recently detected from this tick in Israel. *A. arboreus* may serve as a potential vector of the virus between populations of the cattle egret, but the chance of it being a vector to man is very unlikely.

Relapsing Fever in Israel: new aspects of an old disease

Assous, Marc Victor

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Tick Borne Relapsing Fever (TBRF) was first described in Israel in 1919 by Nicholson and its complete clinical description was published by Adler in 1937. *Ornithodoros tholozani* has been described as the vector and as the causative agent *Borrelia persica*. The diagnosis was based on microscopic examination (thin and thick Giemsa blood smears) and experimental studies were conducted using animal model (mouse, rat and guinea-pig). In the 60 subsequent years, there was no major evolution in the knowledge on this disease. In the year 2000, several research teams in Israel renewed their interest in the subject using bio-molecular methods. The sensitivity and the specificity of microbiological diagnosis can be improved with the help of PCR methods, targeting *flaB*, *16s rRNA* and *glpQ* genes. The use of *flaB* for taxonomic analysis allows the description of a new cluster that corresponds to the Eurasian strains. In addition, 3 sub-types were described in Israel according to the amino-acid sequences of the *flaB* gene. Two new types were also described, by sequencing *flaB* of one strain from Iran and one from the Uzbekistan-Tadjikistan region. *O. tholozani* ticks collected using CO_2 traps have been investigated using *flaB* PCR to determine their infection rates. The number of ticks collected ranged from several to thousands per sample. Infection rates were very variable, ranging from less than 2% to 40%. For single exposures to the vector, the classical pre-exposure treatment is based on doxycycline; it was also reported for post-exposure prevention. However, this drug has a lot of side effects. Therefore, we have empirically used, with success, amoxicillin for ourselves for both pre and post-exposure treatment.

Vector-borne diseases around Israel

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The epidemiology of vector-borne diseases is changing in the recent years. These diseases which were once limited to the tropical regions of the world seems to emerge in temperate zones, due to multiple factors, such as climate change, changes in agricultural land use, animal transportation , tourism and population density. Viral diseases, namely Crimean-Congo Hemorrhagic fever, Rift-valley fever, Chikungunya and Dengue are examples of diseases which emerge in various manners and regions. Crimean-Congo hemorrhagic fever (CCHF), zoonotic tick-borne disease caused by the genus nairovirus, family Bunyaviridae, demonstrates the greatest distribution of all tick-borne viruses, being endemic in parts of Eurasia and Africa. Possible human-to-human transmission increases the likelihood of dispersion. The recent outbreaks in Turkey and the Balkans emphasize its importance in our region. Rift –valley fever, zoonotic and mosquito-borne viral disease (phlebovirus, Bunyaviridae), was confined to the African continent until the year 2000, when it emerged in the Arabian Peninsula. The factors responsible for this massive outbreak are not fully elucidated. Epizootics affecting the animal reservoir (cattle, sheep, goat) usually precedes human illness. Chikungunya is a viral disease (alphavirus, Togaviridae) transmitted by Aedes mosquitoes. It is a specifically tropical disease. Human beings serve as the Chikungunya virus reservoir during epidemic periods. Outside these periods the main reservoir are monkeys, rodents and birds. An ongoing epidemic in the Indian Ocean affected 266,000 of the 775,000 inhabitants in Reunion. Recently (2007), the virus emerged in Italy. Dengue viruses (1-4) (Flaviviridae) are the most important arbovirus human pathogens, and are also unusual as they use humans as reservoir hosts. Both the Dengue and the Chikungunya viruses share the same vectors (*Aedes aegypti*, *Aedes albopictus*). This fact explains the mixed epidemics which have occasionally been described. Warmth and humidity favor the density and dispersion of the vectors, whether ticks or mosquitoes. Accumulating evidence of other vector-borne diseases such as west-Nile fever introduction into North America, East Asian strains of tick-borne viral encephalitis now circulating in Europe, and Usutu virus emergence in Austria show that vector-borne viruses change their "classic" characteristics, and may reach new and unpredictable regions.

Sunday, December 27, 2009

14.00 – 15.30 **Session 3: Tick-borne diseases II**

Chairpersons: David Hasin & Yuval Gottlieb

14.00 – 14.20 **Keysary, Avi. Spotted Fever in Israel – What's new?**

14.20 – 14.40 **Meltzer, Eyal, Eyal Leshem, Drorit Atias & Eli Schwartz. Tick-borne infections in Israeli travelers**

14.40 – 15.00 **Harrus, Shimon. Rickettsial diseases in pet animals in Israel**

15.00 – 15.20 **Shkap, Varda. Bovine anaplasmosis - insights on disease transmission**

Spotted Fever in Israel – What's new?

Avi Keysary

Israel Institute for Biological Research, Dpt. Infectious Diseases, Ness-Ziona, Israel

Mediterranean spotted fever (MSF), a tick-borne disease caused by *Rickettsia conorii*, is endemic in Israel. The main vector of MSF in the Mediterranean area is the brown dog tick *Rhipicephalus sanguineus*. The incidence of the disease in recent years is 22-49 cases per year. Diagnosis is routinely done by serology. PCR tests are used for diagnosis in the acute phase of the diseases, usually for near-fatal or fatal cases. Recently, two other pathogenic members of the spotted fever rickettsiae group – *R. aeschlimannii* and *R. massiliae* - were detected in ticks from a variety of indigenous wildlife.

Tick-borne infections in Israeli travelers

Meltzer, Eyal, Eyal Leshem, Drorit Atias & Schwartz Eli

The Sheba Medical Center, Tel Hashomer, Israel

A few tick-borne diseases are endemic to Israel, including *Rickettsia connorii* and *Borrelia persica* (the causative agent of Relapsing fever) infections. However, travel related tick-borne infections are increasingly seen. In recent years, travelers with proven tick borne infections were diagnosed at the Center for Geographical Medicine at the Sheba Medical Center. The majority were cases of African tick bite fever (ATBF), mostly acquired in Africa. ATBF occurred mostly in travelers returning from Safari tours to South Africa, with an incidence that varied from 1-25% between groups. Cases presented with a typical eschar, an acute febrile illness and vesicular rash, and outcome was favorable in all cases. In addition, 3 cases of *R. tsutsugamushii* were diagnosed in travelers to India – all three presented with a severe multisystem disease which required intensive care. Among travelers returning from developed countries in North America and Europe, several cases of Lyme disease were seen. All cases were diagnosed clinically, with serological confirmation in most cases. Unfortunately, diagnostic delay was frequently seen due to lack of diagnostic methods for many of the imported tick borne infections in Israel. An increase in physicians' awareness of imported tick borne infections and an extended diagnostic infrastructure are both urgently required.

Rickettsial diseases in pet animals in Israel

Harrus, Shimon

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Rickettsial organisms are responsible for a number of important and potentially fatal infectious diseases of companion animals. The most prevalent rickettsial pathogen of companion animals in Israel is *Ehrlichia canis*. However, pets in this region are exposed to other rickettsial pathogens including *Anaplasma platys, Rickettsia conorii* and *Rickettsia felis*.

Ehrlichia canis: *Ehrlichia canis* is the etiologic agent of canine monocytic ehrlichiosis (CME). It is the most common infectious disease affecting dogs in Israel. The rickettsia is transmitted by the brown dog-tick, *Rhipicephalus sanguineus*. Common clinical signs include lymphadenomegaly, splenomegaly, dermal and mucosal petechiae and ecchymoses, and epistaxis. Common hematological signs include thrombocytopenia and pancytopenia. Hypoalbuminaemia and hyperglobulinaemia are common biochemical abnormalities. Diagnosis of the disease is challenging due to its different phases and multiple manifestations. The disease is not zoonotic, however a closely related strain, named "Venezuella canine ehrlichia" have been shown to infect humans. A recent study investigating prognostic indicators for the disease has shown that pronounced pancytopenia (WBC<4 x 10^9/L; HCT<25%; PLT<50 x 10^9/L) was as a risk factor for mortality. Moreover, 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%. These prognostic indicators can be easily obtained at presentation, are inexpensive, and may be useful aids when treatment and prognosis are being considered.

***Anaplasma platys*:** *Anaplasma platys*, formerly known as *Ehrlichia platys*, is the cause of infectious canine cyclic thrombocytopenia (ICCT). It is transmitted by the brown dog-tick, *Rhipicephalus sanguineus.* The disease may manifest as a subclinical or as an acute clinical disease. The latter form of the disease with distinct clinical signs has only been described in Greece and Israel, and may be attributable to a more virulent strain of *A. platys*, in the Mediterranean region. The clinical signs include pale mucous membranes, fever and lymphadenomegaly. The main hematological and biochemical findings are thrombocytopenia and the presence of giant platelets, anemia, monocytosis and lowered albumin concentrations. Uveitis has been associated with *A. platys* in natural infection.

Rickettsia felis: *Rickettsia felis* is an emerging bacterial pathogen and the cause of flea-borne spotted fever. Cat fleas (*Ctenocephalides felis*), collected from cats in Israel, were screened in 2006 for the presence of *R. felis* by polymerase chain reaction and sequencing of 5 different genes (*gltA, fusA, ompA, ompB, 17-kDa*). *Rickettsia felis* DNA was detected in 7.6% of the flea pools (5-20 fleas per pool). This was the first detection of *R. felis* within its vector in Israel and the Middle East. Although no clinical cases have been reported in humans in Israel to date, these findings suggest that this infection is prevalent in Israel.

Rickettsia conorii: *Rickettsia conorii*, the etiologic agent of Mediterranean spotted fever, was associated with an acute, febrile illness in 3 male Yorkshire terriers from

Sicily. The prevalence of IgG-antibodies reactive with an Israeli strain of *Rickettsia conorii* (Israeli strain # 487), was examined in humans and dogs from 2 rural villages in Israel where the disease has been reported in humans. Sixty-nine of 85 (81%) canine sera and 14 of 136 (10%) of human sera had anti-*R. conorii* antibodies. No direct association could be made between seropositivity of people and ownership of a seropositive dog. 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. These results suggest that dogs can serve as effective sentinels for *R. conorii*.

Bovine anaplasmosis - insights on disease transmission

Shkap, Varda

Division of Parasitology, Kimron Veterinary Institute, Bet Dagan, 50250, Israel

Bovine anaplasmosis caused by the intraerythocytic rickettsia *Anaplasma marginale*, is one of the most significant economic hindrance to the livestock industry. The disease is enzootic to nearly half the world's livestock production. Anaplasmosis generates severe losses through mortality, reduced weight gains and decreased milk production. Successful control of anaplasmosis is based on immunization of cattle with live *A. centrale*, causing relatively mild or subclinical disease. The two organisms are genetically distinct based on the 16S rRNA sequences, and there are clear differences in other genes, antigens and virulence. There are about 20 tick species incremented in transmission of anaplasmosis, and marked differences in transmissibility have been identified in genetically distinct *A. marginale* strains. The tick gut tissues and salivary glands were clearly demonstrated to be crucial sites for development of *A. marginale* in vector-to-cattle transmission, but the requirements for efficient biological transmission of *A. centrale* are not known. In the present study transmission of field strains of *A. marginale* and of the *A. centrale* vaccine strain to susceptible calves was examined. Two genetically distinct *A. marginale* Israeli field strains, tailed and non-tailed (AmIsT and AmIsNT, respectively), were efficiently transmitted by *Rhipicephalus sanguineus*, whereas *Hyalomma excavatum* transmitted only the tailed *A. marginale* isolate. The *R. (Boophilus) annulatus* tick did not transmit *A. marginale*. None of the three tick species transmitted *A. centrale*. By means of *msp1a* primers similar size amplicons were amplified in PCR assays from either *A. marginale*-infected calves that were used for acquisition feeding, from *R. sanguineus* fed on the infected calves, or from calves to which anaplasmosis had been successfully transmitted by these ticks. Although an *A. centrale*-specific fragment was amplified from salivary glands of *R. sanguineus*, no transmission to susceptible cattle occurred during 3 months of observation, and anaplasmosis was not induced in splenectomized calves that were sub-inoculated with blood from calves from which *R. sanguineus* had fed.

Monday, December 28, 2009

08:30 - 09:00 **Registration**

09.00 – 10.30 Session 4: Plenary Lectures

Chairpersons: Michal Shapira & Moshe Efrat

09.00 – 09.30 **Balicer, Ran & Itamar Grotto. Swine Flu (Pandemic A/H1N1) as a model for a 21'st century emerging infectious disease**

09.30 – 09:50 **Golenser, Kobi. Malaria: from monkeys to human – closing the circle**

09.50 – 10.30 Human African trypanosomiasis in an Israeli traveler
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a. Meltzer, Eyal, Eyal Leshem, Daphna Gutman & Eli Schwartz. Human African trypanosomiasis in an Israeli traveler

b. Goldshmidt, Hanoch, Devorah Matas, Anat Kabi, Shai Carmi, Ronen Hope & Shulamit Michaeli. A potential new target for trypanosomiasis drug therapy

Swine Flu (Pandemic A/H1N1) as a model for a 21'st century emerging infectious disease

Balicer, Ran[1,2], MD, Ph.D., MPH & Itamar Grotto, MD, Ph.D., MPH[2,3]

[1]Clalit Health Services; [2]Epidemiology Department, Ben-Gurion University; [3]Public Health Services, Israel Ministry of Health

The 2009 influenza pandemic has swept through the world in a matter of months, with an increasing death toll and significant economical disruption to date. This outbreak serves as a model for understanding the 21'st century emerging infectious diseases – their characteristics and the factors which impact our ability to confront them. We will discuss the attributes of the current outbreak and key lessons learned at this early point.

Malaria: from monkeys to human – closing the circle.

Golenser, Jacob

Department of Microbiology and Molecular Genetics, The Faculty of Medicine, Hadassah Medical School, The Hebrew University of Jerusalem, Jerusalem, Israel

Julius Wagner-Jauregg, received the 1927 Nobel Prize for using a monkey strain, *Plasmodium knowlesi* for malaria therapy of syphilis. Transmission of *P. knowlesi*, from human to human was by blood passage. In 1960, Eyles et al. demonstrated the first experimental mosquito transmission of a simian malaria organism to humans *(P. cynomolgi)*. Later, *P. inui* and *P. knowlesi* (Chin et al. 1967) were also transmitted from monkeys to humans by mosquitoes. However, the resulting human infections were not considered until recently as having an epidemiological significance. During the last 5 years *P. knowlesi* has become a significant cause of potentially severe malaria in South East Asia. The distribution has spread to Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam. *P. knowlesi* malaria in humans is often misdiagnosed as *P. malariae*. The trophozoite, schizont, and gametocyte stages of *P. knowlesi* are morphologically indistinguishable from those of *P. malariae* by microscopy, and uncomplicated cases of *P. knowlesi* respond to chloroquine. However, *P. malariae* is associated with a relatively low parasite load and a benign clinical course. In contrast, the 24-h asexual life cycle of *P. knowlesi* is the shortest of all known human and non-human primate malarias. Daily schizont rupture, with attendant fever spikes and a potentially rapid increase in parasite load, is unprecedented in human malaria, and therefore even a short delay in accurate diagnosis, treatment, and adjunctive management could increase the risk of complications. Moreover, the misdiagnosis of *P. knowlesi* malaria and the assumption that *P. malariae* malaria is benign may cause the clinician to look for alternative causes of vital-organ dysfunction. In addition to the problems of delayed and inappropriate management, there are epidemiological implications, specifically those relating to underestimation of the incidence of severe *P. knowlesi* malaria (Cox-Singh et al., 2007; White, 2007). Co-existence of the plasmodium within the erythrocyte is a result of a long evolution. This is the reason for human malaria to be transmitted from human to human. However, most

emerging diseases exist within a host and parasite space including wildlife, domestic animal, and human populations. Therefore, *Plasmodium knowlesi* might be in the future the fifth human malaria parasite and malaria would then be a significant zoonotic disease.

Human African trypanosomiasis in an Israeli traveler

Meltzer, Eyal, Eyal Leshem, Daphna Gutman & Eli Schwartz

Sheba Medical Center, Tel Hashomer, Israel

Human African trypanosomiasis (HAT) has not been previously seen in Israeli travelers. A case of *T. rhodesiense* HAT, which was acquired during a Safari trip to Tanzania was diagnosed in a 31 years old Israeli female traveler. The recognition of suggestive clinical features, including the fever pattern and specific skin lesions, enabled the establishment of the diagnosis, despite multiple negative tests. Due to the increase in reports of HAT in travelers, physicians in the developed world need to be aware of this diagnosis, and to include it in the differential diagnosis of febrile diseases in travelers returning from Sub Saharan Africa.

A potential new target for trypanosomiasis drug therapy

Goldshmidt, Hanoch, Devorah Matas, Anat Kabi, Shai Carmi, Ronen Hope & Shulamit Michaeli

The Mina and Everard Goodman Faculty of Life Sciences, and Advanced Materials and Nanotechnology Institute, Bar-Ilan University, Ramat-Gan 52900 Israel

Trypanosomes are the causative agent of major parasitic diseases such as African sleeping sickness, leishmaniasis and Chagas' disease affecting millions of people mostly in developing countries. These organisms diverged very early from the eukaryotic linage and possess unique molecular mechanisms such as *trans*-splicing and RNA editing. Trypanosomes lack polymerase II promoters that govern the transcription of protein coding genes. Eukaryotes respond to unfolding of proteins in the endoplasmic reticulum (ER) by a distinct transcriptional programming known as the unfolded protein response (UPR). In this study, we demonstrate that despite the lack of transcriptional regulation, trypanosomes change their transcriptome as a response to ER stress by differential mRNA stabilization. Prolonged ER stress induces a unique process, the spliced leader RNA silencing (SLS) that shuts off the *trans*-splicing and the production of all mRNAs. SLS is induced both by prolonged ER stress and by knock-down of factors involved in ER translocation. SLS induces programmed cell death (PCD) evident by the hallmark of apoptosis in metazoa (DNA fragmentation, membrane flipping and ultrastructural changes). We propose that SLS serves as a unique death pathway replacing the conventional caspase-mediated PCD observed in higher eukaryotes. Induction of SLS can potentially be used as a novel drug target for killing trypanosmatid parasites.

Monday, December 28, 2009

11.30 – 13.00 **Parallel sessions (Travel Medicine and General Parasitology)**

11.30 – 13.00 **Session 5: Travel Medicine**

Chairpersons: Michael Dan & Michal Chowers

11.30 – 11.50 **Alon, Danny, Pnina Shitrit & Michal Chowers. Risk behaviors and spectrum of diseases among older travelers: A comparison of young and older adults**

11.50 – 12.10 **Leshem, Eyal, Yehezkel Caine, Eli Rosenberg, Yoram Maaravi, Hagai Hermesh & Eli Schwartz. Tadalafil and Acetazolamide versus Acetazolamide in altitude sickness prevention**

12.10 – 12.30 **Gottesman, Giora. Human Papiloma Virus (HPV) vaccine; also for travelers?**

12.30 – 12.45 **Anders, Gerlind. New developments in epidemiology of *Neisseria meningitidis* and vaccination against invasive meningococcal diseases**

12.45 – 13.00 **Anis, Emilia, Paul E. Slater, Ran Balicer, Daniele Goldmann, Shepherd Roee Singer & Itamar Grotto. Novel Swine-Origin Influenza A (H1N1) 2009: a case of probable in-flight transmission**

Risk behaviors and spectrum of diseases among older travelers: A comparison of young and older adults

Alon, Danny, Pnina Shitrit, Michal Chowers

Infectious Diseases Unit, Meir Medical Center, Kfar Saba

Background: Elderly travel to the developing world is increasing. Little information is available regarding risk behaviors and health during and after travel in this population.
Methods: We compared the risk factors and occurrence of travel-related diseases in two populations of Israelis, travelers aged 60 years and older and travelers in the age group of 20-30 years. Only people traveling for less than a month were included. Pre-travel, each person received routine counseling regarding travel-associated health risks, was immunized, and given anti-malarial prescriptions as needed. Travelers were surveyed by telephone six-to-twelve months following travel about underlying medical conditions, current medications, and travel history. Risk and preventive behaviors, compliance with anti-malarial prophylaxis and history of illness during and after travel were assessed.
Results: Of patients who visited the clinic from January to June 2008, 191/208 (91%) travelers aged 60 and older and 203/291 (69%) travelers aged 20-30 years were contacted by phone and recruited. Fewer elderly travelers drank open drinks, compared to young travelers (8% vs. 35%, $p<0.01$) and fewer purchased street food compared to young travelers (16.2% vs. 37.9%, $p<0.01$). More elderly travelers were fully compliant with their anti-malarial chemoprophylaxis regimen (60.7% vs. 33.8% $p<0.01$). More elderly travelers took organized tours (61% vs. 2%, $p<0.001$). Young travelers more often backpacked (50.7% vs. 10.4%, $p<0.001$). Illness, most commonly diarrhea was reported by 18.8% of elderly travelers compared to 34.0% of the young travelers ($p=0.001$). In a logistic regression model only travel to East Asia and traveling under basic conditions remained significantly associated with illness, irrespective of age.
Conclusions: Because elderly travelers tend to comply with health-related recommendations better and use less risky travel modes, their risk for illness during travel was lower. Traveling to East Asia and travel mode are associated with illness during travel, irrespective of age.

Tadalafil and Acetazolamide versus Acetazolamide in altitude sickness prevention

Leshem, Eyal[a], Yehezkel Caine[b], Eli Rosenberg[c], Yoram Maaravi, Hagai Hermesh[d], Eli Schwartz[a]

[a]Center for Geographic Medicine and Internal Medicine C, Sheba Medical Center, Tel-Hashomer, Israel; [b]Herzog Hospital, Jerusalem, Israel; [c]Department of Occupational Medicine, Ministry of Health, Jerusalem, Israel; [d]Geha Mental Health Center Hospital, Petah Tikva, Israel

Introduction: Acute Mountain Sickness (AMS) and complications including high altitude cerebral edema (HACE) and high altitude pulmonary edema (HAPE) occur when acclimatization is insufficient and are aggravated by exhaustion. Acetazolamide is well accepted for both prophylaxis and treatment of AMS. Phosphodiesterase-5 inhibitors (PDE-5 inhibitors) are a group of drugs proven to be effective in the treatment of pulmonary hypertension. The suggested mode of action of PDE-5 inhibitors (such as Viagra, Cialis) is by reducing pulmonary hypoxic vasoconstriction and hypoxia induced changes in the function of the right ventricle. Recent studies have shown the efficacy of PDE-5 inhibitors in increasing exercise capacity during hypoxia at the Mt. Everest base camp. Sildenafil (Viagra) might also be useful in preventing HAPE, one of the life threatening complications of AMS. It has been proposed that Sildenafil produces its effect at high altitude by reducing hypoxic pulmonary hypertension. Sildenafil has not yet been tested in AMS prophylaxis. The aim of the study was to clinically evaluate the efficacy of Tadalafil (Cialis) in the prevention of AMS, HACE and HAPE and in improving exercise capacity measured by successfully summiting a high altitude peak.

Methods: An open label controlled study comparing tadalafil and acetazolamide versus acetazolamide alone in prevention of altitude illness. Trekkers participating in group efforts to summit Mt. Kilimanjaro and Mt. Kenya were enrolled. Results were evaluated using the Lake Louise AMS scoring system. AMS symptoms were self evaluated according to the Lake Louise questionnaire and evaluated by the group doctor.

Results: The study enrolled 49 participants in 4 groups (3 to Mt. Kenya and 1 to Mt. Kilimanjaro). The mean age of participants was 47±12.4 years (The median age of participants was 50±12.4 years) and 8/49 (16%) were females.

Overall the Tadalafil group had a lower average Lake Louise score (3±2.11 vs. 4.11±4.18). The Tadalafil group also had lower rates of AMS (54% vs. 59%), HACE (5% vs. 18%) and HAPE (0% vs. 7%) as compared with the control group. Successful summit rates were higher in theTadalafil group (95% vs. 88%). None of the results reached statistical significance.

Conclusion: Our results suggest that Tadalafil may be useful in prevention of AMS and severe AMS complications as well as in improving exercise capacity at high altitude.

Human Papiloma Virus (HPV) vaccine; also for travelers?

Gottesman, Giora

Pediatric Infectious Diseases, Meir Hospital

Worldwide 630 million people are currently infected with Human Papillomavirus (HPV). The Prevalence of HPV in sub-Saharan Africa is 3 times than in the USA. Currently there are 20,000,000 cases in the USA. More than 80% of women have HPV by the age of 50. Among Jewish women at all and especially Jewish women in Israel, the prevalence of cancer of the cervix is much lower than in non Jewish. Approximately 130 HPV types have been identified; type 16 and 18 associated with 67% of cervical cancer, types 6 and 11 are responsible for causing over 90% of all anogenital warts. Human papillomavirus (HPV)-related diseases include cancers, low-grade neoplasia, genital warts, and recurrent respiratory papillomatosis. Since 2006, 2 HPV vaccines are widely marketed internationally. Using recombinant technology, both are prepared from purified L1 structural virus-like particles (VLPs). Clinical trials in young women who previously had not been exposed to the targeted HPV types demonstrated nearly 100% vaccine efficacy in preventing cervical precancers, vulvar and vaginal precancers, and genital warts caused by the four vaccine types. New Postlicensure Safety Surveillance published in the JAMA demonstrate that most of the adverse events following immunization rates were not greater than the background rates compared with other vaccines, but there was disproportional reporting of syncope and venous thromboembolic events. Differences between the two vaccines will be highlighted. There are no inherent risks for travelers. HPV is ubiquitous and common worldwide. Risk depends on the behavior of the traveler. Travelers should be given information about the risks of HPV and other sexually transmitted infections.

New developments in epidemiology of *Neisseria meningitidis* and vaccination against invasive meningococcal diseases

Anders, Gerlind

Novartis Vaccines and Diagnostics, Marburg, Germany

Meningococcal disease causes severe morbidity and death, particularly in infants. Epidemiology is dynamic and geographically variable. The most relevant meningococcal serogroups are A, B, C, W-135 and Y. Serogroup A is prevalent in Africa's 'meningitis belt'; B and C are prevalent in Europe, Australasia, and the Americas; W-135 causes outbreaks in the Middle East and Africa; and Y is a concern in North America. No "universal" vaccine protects against all five major serogroups. Childhood and adolescent vaccination with meningococcal C conjugate vaccines has drastically decreased serogroup C disease in many countries. Quadrivalent (A, C, W-135, Y) polysaccharide and glycoconjugate (using diphtheria toxoid as carrier protein) vaccines are not licensed in the highest risk population, the under-twos. Phase II and III trials of a novel investigational MenACWY-CRM conjugate have shown this investigational vaccine is well tolerated and highly immunogenic for all four serogroups from 2 months of age in a range of schedules. Compared with existing polysaccharide vaccines, conjugate vaccines provide a favorable immunogenicity and do not show hyporesponsiveness. A separate investigational Men B vaccine containing recombinant protein antigens developed using 'reverse vaccinology' has shown satisfactory tolerability and immunogenicity in Phase II trials in adults, and in infants. If and when licensed, these investigational vaccines will provide important tools to help control meningococcal disease caused by all the major serogroups in the age populations at high risk around the world.

Novel Swine-Origin Influenza A (H1N1) 2009: a case of probable in-flight transmission

Anis, Emilia, MD, MPH, Paul E. Slater, MD, MPH, Ran Balicer, MD, MPH, PhD, Daniele Goldmann, MD, MPH, Shepherd Roee Singer MD, MPH & Itamar Grotto MD, MPH, PhD

Ministry of Health, Jerusalem, Israel

We describe a case of A (H1N1) 2009 Influenza in an Israeli traveler returning from Central America in April, 2009. Epidemiological investigation points to in-flight transmission as the most likely cause. In-flight transmission of influenza and other contagious diseases remains a real and growing concern in a world of increasing globalization. Our recommendation to the general population to be vaccinated against pandemic flu reduces the risk of in-flight transmission and the unpleasantness of suffering from influenza while traveling.

Monday, December 28, 2009

11.30 – 13.00 **Parallel sessions (Travel Medicine and General Parasitology)**

11.30 – 13.00 **Session 6: General Parasitology**
Chairpersons: Varda Shkap & Daniel Gold

11.30 – 11.45 Berlin, Dalia, Abedelmajeed Nasereddin & Gad Baneth. *Trypanosoma evansi* infection in horses and camels in Israel – What have we learned?

11.45 – 12.00 **El-On, Joseph, Ruth Snier, Michael Albeck, Revital Duvdevani & Benjamin Sredni. Efficacy of the immunomodulator AS-101 on *Leishmania major* development in vitro in macrophages and in vivo in experimentally infected mice**

12.00 – 12.15 **Kovalenko, Dmitriy A., Shavkat A. Razakov, Evgeny N. Ponirovsky, Alon Warburg, Rokhat M. Nasyrova, Valentina I. Ponomareva, Aziza A. Fatullaeva, Lionel F. Schnur, Charles L. Jaffe, Gabriele Schönian & Gad Baneth. Canine leishmaniasis in Eastern Uzbekistan: its relationship to human visceral leishmaniasis and the sand fly fauna of the region**

12.15 – 12.30 **Mazuz, Monica L., Lea Fish, R. Wolkomirsky, B. Leibovich, D. Reznikov, I. Savitsly, Theah Molad & Varda Shkap. Vaccination of cows against *Neospora caninum* – associated abortions with live parasites**

12.30 – 12.45 **Ment, Dana, Galina Gindin, Alice Churchill, Itamar Glazer, Asael Rot & Michael Samish. Evaluation of host-pathogen interactions of a fungal arthropod pathogen – ultra structural observations**

12.45 – 13.00 **Rot, Asael, Michael Samish, Galina Gindin, Dana Ment & Itamar Glazer. The effect of entomopathogenic fungi on ticks' behavior**

13.00 – 14.00 **Lunch**

Trypanosoma evansi infection in horses and camels in Israel – What have we learned?

Berlin, Dalia [1], Abedelmajeed Nasereddin[2,3] & Gad Baneth[1]

[1]Koret School of Veterinary Medicine Teaching Hospital, Hebrew University of Jerusalem, Israel; [2]Kuvin Centre for the Study of Tropical and Infectious Diseases, IMRIC, Hebrew University-Hadassah Medical School, Jerusalem, Israel; [3]Al-Quds University, Leishmaniasis Research Center, Abu-Deis, The Palestinian Authority

Trypanosoma evansi is an insect-borne protozoal pathogen that infects mainly equids, camels and buffalo. It is the cause of the disease known as Surra which is characterized by chronic weight loss, limb edema, anemia, icterus, neurological abnormalities and in some cases abortions and death. Following the first diagnosis of *T. evansi* infection in a horse in Israel in 2006, in the southern Dead Sea area, and an outbreak of the disease in another horse and 5 camels in the farm from which this horse originated, a country wide survey for the prevalence of anti-*T. evansi* antibodies in the horse population was initiated. The survey took place between October 2007 and December 2008. Blood samples were collected for packed cell volume determination, blood smear evaluation, and serology. The card agglutination commercial kit, CATT/*T. evansi*, was used for the serology. The country was divided into 7 regions: the Golan Heights and Hula valley, Jezereel valley and Lower Galilee, Coastal area, Inner plain (Shfela), Jerusalem area, Northern Negev, and the Arava and Dead Sea. In total, 610 horses were sampled from 60 stables. None of the examined horses exhibited overt clinical signs of Surra. The overall country sero-prevalence was 4.43% (27/610). The region with the highest sero-prevalence was the Coastal region with 8% (12/150) and the region with the lowest prevalence was the Jerusalem region with no sero-positive animals (0/25). Parasitemia was not detected in any of the blood smears of the sero-positive animals and none of these horses had anemia. Since trypanosome parasitemia is sometimes difficult to detect on blood smears, PCR followed by the reverse dot blot (RDB) technique were implemented on DNA extracted from some of the blood samples. Most of the samples from the Arava and Dead Sea region and most of the sero-positive samples from the other regions were examined. Eight of the 18 sero-positive samples that were examined by RDB were positive. In the Arava and Dead Sea region, 25 of the 133 (19%) samples examined were RDB-positive while 4 of them were also sero-positive. A second sampling was conducted in the Arava and Dead Sea region only, one year after the first sampling (October 2007 and October 2008), in order to investigate changes in the sero-prevalence in the population over time. In this survey, 119 horses were sampled in comparison to 135 in the first survey. In the second survey of this region, the sero-prevalence was lower that in the first survey, 4.2% (5/119) and 5.9% (8/135) respectively. Only 2 horses were sero-positive in both sampling dates. An additional study was begun in September 2009 in order to investigate the sero-prevalence of the camel population in the Negev and Arava region. So far, 105 camels were sampled in the Negev region of which only 1 was sero-positive without detectable parasitemia. Since positive serology and positive RDB did not tend to be associated with clinical signs of Surra in these surveys, it might be concluded that *T. evansi* infection in horses and camels has a limited impact on these populations in most areas of the

country. Nevertheless, further investigation is warranted into the epidemiology of the disease and the sensitivity and specificity of the diagnostic methods.

Efficacy of the immunomodulator AS-101 on *Leishmania major* development *in vitro* in macrophages and *in vivo* in experimentally infected mice

El-On, Joseph[1], Ruth Snier[1] Michael Albeck[2] Revital Duvdevani and Benjamin Sredni[3]

[1]The Shraga Segal Dept. of Microbiology and Immunology, Ben-Gurion University of the Negev and Laboratory of Parasitology, Soroka University Medical Center, Beer Sheva, [2]Department of Chemistry, and [3]Department of Life Sciences, Bar Ilan University, Ramat Gan, Israel

The efficacy of AS-101 [ammonium tri-chloro(dioxoethylene-O,O'-) tellurate] (BioMAS, Kfar Saba, Israel) a novel immune-response-activating agent, either alone or combined with paromomycin (PR)/methylbenzethonium chloride (MBCL) on *Leishmania major* development *in vitro* and *in vivo* in experimentally infected mice was determined. AS-101 was highly effective against *Leishmania* promastigotes (IC_{50}=8μg/ml) and amastigotes (IC_{50}=0.1 μg/ml) *in vitro*. On the 3rd day of treatment, AS-101 at 0.5, 5, and 100 μg/ml inhibited the intracellular amastigotes development by 65.9%, 80.2%, and 99.4%, respectively. Approximately 1000 times higher concentration was required to induce a toxicity to the host cell – the macrophage (ID_{50}>100 μg/ml). AS-101 was found to be almost as effective as the gold standard drug paromomycin (IC_{50}=40 μg/ml) against *L. major* amastigotes *in vitro*. Combination chemotherapy of AS-101 and PR showed an additive effect. Treatment of infected Balb/c mice with 1% AS-101 ointment (AS-ointment), starting 8 wks after infection with $1x10^5$ promastigotes showed variable results. A significant decrease in parasites load and a delay in mice mortality was observed with AS-ointment and Leshcutan (15% PR + 12% MBCL in soft white paraffin), applied sequentially to the lesion, once daily, for 20 and 5 days, respectively. A lower efficacy was observed with AS-ointment, containing PR (50mg/ml) plus MBCL (40mg/ml), applied once daily for 5 days followed by AS-ointment given alone for additional 15 days. AS-101 alone, either applied topically (AS-ointment) or administrated intraperitoneally (250μl =30μg) for 20 days to infected Balb/c mice showed only partial effect. The present study suggests that a combination of AS-101 and Leshcutan (and possibly other anti-leishmanial compounds) is highly beneficial in the treatment of CL caused by *L. major* in experimentally infected mice. However, for final conclusions, various drug concentrations and various mode of treatments should be further examined in both susceptible (Balb/c) and resistant (CBA, C3H, C57BL/10) mice.

Canine leishmaniasis in Eastern Uzbekistan: its relationship to human visceral leishmaniasis and the sand fly fauna of the region

Kovalenko, Dmitriy A.,[1] Shavkat A. Razakov[1,] Evgeny N. Ponirovsky[2], Alon Warburg[3], Rokhat M. Nasyrova[1], Valentina I. Ponomareva[1], Aziza A. Fatullaeva[1,] Lionel F. Schnur[3], Charles L. Jaffe[3], Gabriele Schönian[4], and Gad Baneth[5]

[1]Isaev Institute, Samarkand, Uzbekistan; [2]Sechenov Medical Academy, Moscow, Russia; [3]IMRIC, Hebrew University - Hadassah Medical, Israel; [4]Charité University, Berlin, Germany; [5]School of Veterinary Medicine, Hebrew University, Israel

The Namangan Region, Pap District, Eastern Uzbekistan is the main focus of visceral leishmaniasis (VL) in this country. In total, 52 cases of human VL were registered during 2001-2008 in this region. A study on the epidemiology of VL in this area was carried out in 2007-2008 and focused on the villages of Chodak, Oltinkon, Guliston and Chorkesar located at elevations of 900-1200 above sea level. A total of 162 dogs and 4 foxes were tested for leishmanial infections. Blood was drawn for serology and PCR. If clinical signs of the disease were present, conjunctival swabs, lesion tissue smears, and aspirates from lymph nodes and the spleen were taken. Forty-two dogs (26%) had clinical signs suggestive of VL. Sixty-five (40%) were sero-positive. ITS-1 PCR was performed for 142 dogs using different types of tissue samples and 42 (28%) of them were PCR-positive. Leishmanial parasites were cultured from lymph node and/or spleen aspirates from 10 dogs. None of the foxes were found to be infected. DNA samples extracted from eight strains isolated from dogs were typed using multi-locus microsatellite typing (MLMT) and multilocus enzyme electrophoretic analysis (MLEE), using a 15 enzyme system. These analyses revealed that the strains belong to the most common zymodeme of *L. infantum*, i.e., MON-1, and form a unique group when compared to MON-1 strains from other geographical regions. Blood samples were taken from 521 children. Anti-leishmanial antibodies were detected in 52 (10%) of the samples. Leishmanial parasites were cultured from bone marrow aspirates in three cases of human VL. Almost 3,000 sand flies were collected during the field trip in July 2007 by direct aspiration from bedroom walls and using CDC light traps and castor-oil sticky traps in and around houses and animal shelters. The species composition of sand flies in rooms and outside homes were similar. *P. sergenti* (55%) was the most prevalent species followed by *P. longiductus* (19%), the putative vector of *L. infantum, P. papatasi* (10%), *P. keshishiani* (7%) and *P. alexandri* (4%). On the sticky papers placed in residential and domestic areas in the villages of Oltinkon and Chodak, *P. sergenti* (77.9%) predominated again followed by *P. longiductus* (10.6%), *P. angustus* (7.3%) and *P. papatasi* (2.4%). More than 7000 sand flies were collected during the field trips in July and September 2008. *P. sergenti* (72.4-87.6%) was the most prevalent species followed by *P. alexandri* (3.2-17.6%), *P. papatasi* (2.4-11.5%), *P. longiductus* (1.3-3.2%), *P. (Adlerius) sp.* (0.2-3%), and *P. angustus* (1-2.4%). *P. longiductus* is the likely vector of *L. infantum* in the Namangan region. The data obtained through this study confirm the existence of an active focus of VL in the Namangan region of Uzbekistan. The facts that *L. infantum* was the causative agent of infection, that only children and not adults were affected by the disease, and that dogs with typical clinical signs of canine VL were encountered, suggested that a zoonotic form of VL similar in epidemiology to Mediterranean VL is

present in Uzbekistan. This study was supported by the European Community INTAS program grant no. 05-1000008-8043.

Vaccination of cows against *Neospora caninum* – associated abortions with live parasites

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Neosporosis caused by the obligatory intracellular protozoan *Neospora caninum*, is one of the major causes of abortion and reproductive failure in cattle worldwide, including Israel. Abortion is the only clinical sign of infection, and infected dams that have not aborted may infect their offspring. The principal and highly efficient route of transmission of neosporosis is via *in utero* infection of the offspring. In Israel serological and molecular studies in zero-grazing dairy cattle revealed that 40% of abortions were associated with *intra uterine* neosporosis. It has been found that during gestation the cellular immune response was physiologically down-regulated, which allows multiplication of the parasite, and recrudescence of infection in chronically infected dams. In the present study, seropositive dams were immunized with live *N. caninum* (Israeli isolate NcIs491) parasites under field conditions, on a farm where there was 33% seroprevalance to *N. caninum*. Doses of 1×10^8 live, cell-free *N. caninum* tachyzoites were administered to zero-grazing, seropositive, pregnant dairy dams at between 120 and 140 days of gestation. A total of 100 dams with specific antibody IFA titers of >1:400 were vaccinated; 47% of them exhibited titers of ≥1:3200. All vaccinates were monitored for pregnancy outcome and antibody level. Following vaccination, incidence of reproductive failure and abortion were similar among immunized and control, non-immunized dams. However, the abortion rate was significantly reduced from 25% in control cows to 18.4% in those immunized at initial titers of ≥1:3200. Some of the calves were serologically negative to *N. caninum*, although born to vaccinated dams. The results obtained showed that vaccination of seropositive cows is feasible and safe, and might significantly reduce losses caused by infection of dairy cattle with *N. caninum*.

Evaluation of host-pathogen interactions of a fungal arthropod pathogen – ultra structural observations

Ment, Dana[1], Galina Gindin[1], Alice Churchill[3], Itamar Glazer[1], Asael Rot[2] and Michael Samish[2]

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The fungus *Metarhizium anisopliae* is known as an efficient biological control agent of insects and ticks. *Metarhizium* spp. can develop as saprophytic fungi in the rhizosphere and on organic matter or as arthropod pathogens. Pathogenesis starts when conidia adhere to a host cuticle. The conidia germinate on the host surface and penetrate the cuticle using a unique structure, the appressorium, which is formed at the tip of the germ tube. The appressorium generates high turgor pressure on the cuticle and secretes cuticle-degrading enzymes, enabling the fungus to penetrate the arthropod body. Inside the host, the fungus develops as short hyphal bodies, which proliferate in the hemolymph, exploiting available nutrients and secreting presumed toxins, which are believed to contribute to the death of the host. Finally, the fungus grows out of the dead host and forms conidia on the surface. Laboratory bioassays demonstrated that different tick stages and species, as well as insects, varied in their susceptibility to *M. anisopliae* fungi. While some hosts were highly susceptible (LT_{50} – 3-7 days), other hosts were resistant to the pathogens. To evaluate the compatibility determinants of these pathogen-host interactions, an ultra structural technique was used. Arthropods were infected with green fluorescent protein (GFP)-expressing fungus and it's development was observed over time with a confocal laser scanning microscope. Fungal growth was observed on all hosts used in the study. However, on resistant hosts, the fungus either developed as a saprophyte, producing a dense mycelial sheath on the surface or GFP-expressing conidia germinated but failed to develop further and, at some point, could no longer be detected on the host surface, suggesting death of the fungus. Penetration into host cavities was observed only on susceptible hosts.

The effect of entomopathogenic fungi on ticks' behavior

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Ticks are obligatory blood sucking Arachnids feeding on vertebrates. Adult Ixodidae ticks feed for days to weeks. Ticks cause toxemia, anemia, and in some cases paralysis to their vertebrate hosts. However, a more serious concern is their ability to serve as vectors of many vertebrate pathogens. The subsequent damage to farm animal health causes significant economic losses. Present tick control is based on the use of chemical insecticides, which may be harmful to the environment. In addition, such compounds are known to be less useful as tick populations develop resistance to them. Ticks have numerous natural enemies and pathogens, but only a few of them have been evaluated for use as potential tick bio-control agents. Entomopathogenic fungi (EPF) are known as effective bio-control agents against many insects. However, only in recent years have scientists started to study the potential use of EPF for tick control. One of the disadvantages of EPF as a pesticide is its slow killing rate. Our previous observations demonstrated that *Metarhizium anisopliae*-7 conidia are highly efficacious in killing all stages of the kennel tick (*Rhipicephalus sanguineus*) including its eggs. In the present study unfed *R. sanguineus* larva and nymph were dipped in M. anisopliae-7 conidial suspension (1×107 conidia/ml), and the ticks' phototropism, negative geotropism and attraction to rodents were recorded. The influence of the fungal infection on the success of unfed adult ticks attachment to mammals was observed as well. The behavior of larvae and nymphs 48 h and 96 h post infection (PI) showed a dramatic reduction in all behavior criteria tested. The attachment success of infected unfed adults was dramatically reduced within 12 d PI. The results demonstrated that fungi-infected ticks significantly changed their normal behavior days before the infected ticks died. The significant changes in infected ticks' behavior shortens the time in which ticks can harm their hosts PI, including reducing the ticks' ability to transmit vertebrate pathogens, thus increasing the effectiveness of anti-tick fungi-based agents.

Monday, December 28, 2009

14.00 – 15.30 **Parallel sessions (Tropical Medicine and Molecular Parasitology)**

14.00 – 15.30 **Session 7: Tropical Medicine**

Chairpersons: Iris Ostfeld & Mik Alkan

14.00 – 14.15 **Schwartz, Eli. Outbreak of Paratyphi A infection in Israeli travelers to Nepal**

14.15 – 14.30 **Sabag, Avi, Emilia Anis, Daniele Goldmann & Eli Schwartz. Malaria in the pediatric traveling population: a nation-wide study**

14.30 – 14.45 **Neuberger, Ami, Eyal Klement, Reyes Grassi, Mauricio Carlos & Alon Stamler. Risk factors for malaria among healthcare workers in Equatorial Guinea - Implications for long term travelers**

14.45 – 15.00 **Wieder-Finesod, Anat, Guy Choshen & Eli Schwartz. Amebic liver abscess among Israeli travelers**

15.00 – 15.15 **Segel, Michael J., Yehudit Rozenmann & Eli Schwartz. Histoplasmosis is returning travelers in Israel: Experience of the Travel Medicine Service, Chaim Sheba Medical Center, Tel Hashomer**

15.15 – 15.30 **Marva, Esther & Tamar Grossman. Laboratory tests for parasitic diseases in Israel**

Outbreak of Paratyphi A infection in Israeli travelers to Nepal

Schwartz, Eli and The Paratyphi A Working Group

Sheba Medical Center, Tel Hashomer, Israel

Typhoid fever, or its inclusive name Enteric fever, is a term for systemic infections caused by *Salmonella enterica*, with both serotype *S. typhi* and *S. paratyphi.* The clinical presentations of the two infections are indistinguishable, with similar treatments and outcomes. In this report, we describe an outbreak in Israeli travelers to Nepal of typhoid fever due to *S. paratyphi* A infection. During the month of October 2009, there were 36 cases of febrile travelers who returned from Nepal diagnosed with Paratyphi A, which was proven in almost all cases by blood culture. During this period of time in Nepal, there were no outbreaks of typhoid fever among non-Israeli travelers to the region or among the local population. The Paratyphi A isolates were found to have the same antibiotic sensitivity pattern. Moreover, pulsed-field electrophoresis gel examination demonstrated that all isolates were identical, thus proving a single source for this outbreak. Tracing back and identifying the restaurants where these patients had eaten revealed that there was indeed one place common to all those infected. All of the patients had eaten at the Chabad House in Pokhara, and over a period of several days and not sharing a single meal. Due to a lack of cooperation from the managers of the Chabad House, the source of the infection, most certainly a typhoid carrier that handled food has not yet been identified. This outbreak reflects most probably the largest ever recorded outbreak of typhoid fever in travelers which can be attributed to a single source ("Typhoid Mary").

Malaria in the pediatric traveling population: a nation-wide study

Sabag, Avi, Emilia Anis, Daniele Goldmann & Eli Schwartz

Sheba Medical Center, Tel Hashomer & Ministry of Health, Jerusalem

Background: Malaria remains one of the most common infectious diseases worldwide, causing over a million deaths annually mostly in Africa and predominantly in children under 5 years old. Most studies of the malaria pediatric population are based on reports from endemic countries, while data from western countries are scarce. The goal of our study was to review all pediatric malaria cases reported in Israel and to examine their outcomes.
Methods: All pediatric (age≤18) malaria cases reported to the Israeli Ministry of Health from 1995 to 2008 were retrospectively examined. The cases were analyzed for epidemiological, clinical and laboratory findings as well as outcomes. Comparisons were made between non-immune and partially-immune children and with a parallel cohort of adult malaria patients.
Result: There were 41 pediatric malaria cases with mean age 10.4 ±5.6, among them 26 (63.4%) were males. Malaria was acquired in Africa in 38 (92.7%) of the cases and falciparum malaria was diagnosed in 18 (43.9 %). Among the falciparum malaria cases, 10 (55%) met the criteria of severe malaria (3 were hospitalized in ICUs, 1 case of ARDS, 6 cases of cerebral malaria and 6 cases of severe anemia). There were no fatalities. There were no severe malaria cases in the non-falciparum malaria group.
No significant difference in the incidence of severe malaria between non-immune and partially-immune children was found, but the rate of severe malaria in children infected with *P. falciparum* was significantly higher (>0.001) compared with young adults (age 18-40).
Conclusions: Young age is a risk factor for severe malaria in children regardless of immune status.

Risk factors for malaria among healthcare workers in Equatorial Guinea - Implications for long term travelers

Neuberger, Ami[1], Eyal Klement[2], Reyes Grassi, Mauricio Carlos[3] & Alon Stamler[4]

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Background: Non-immune long-term travelers to sub- Africa are at a high risk of contracting malaria. Most previous studies described risk factors in short-term travelers, and did not determine the spatial distribution of malaria cases.
Methods: We conducted a cohort study evaluating the risk factors for malaria among health-care personnel working in a hospital in Bata, Equatorial Guinea. Demographic data was recorded for all workers, and the spatial distribution of malaria cases within the hospital perimeters was determined.
Results: During 2008 non-complicated falciparum malaria was diagnosed in 15/104 workers (14.4%). In a multivariate analysis acquiring malaria was associated with living on the first floor of all buildings in the hospital ground (0.004), but not with the distance from the presumed mosquito breeding site (p=0.82). Smoking was also associated with the risk of acquiring malaria (p=0.035). Low compliance with chemoprophylaxis, barrier clothing and the use of mosquito repellant was observed in both groups. Chemoprophylaxis was associated with a decreased risk of acquiring malaria in a univariate analysis (p=0.02), but not in the multivariate analysis (p=0.11).
Conclusions: living in the ground floor of apartment buildings in Sub-Saharan Africa, as opposed to living in higher floors, confers an increased risk of acquiring malaria. Smoking also increases the risk of malaria, probably through environmental exposure. The compliance of health-care workers with malaria prophylaxis is extremely low, as was previously described for other long-term residents.

Amebic liver abscess among Israeli travelers

Wieder-Finesod, Anat,Guy Choshen & Eli Schwartz

Infectious Disease Unit and the Center of Geographic Medicine & Tropical Diseases, Tel Aviv University, Sheba Medical Center, Tel Hashomer, Israel

Background: Amebiasis is an infection with *Entamoeba histolytica*. This parasite has a high prevalence in poor socioeconomic countries. In resource-rich nations, infections may be seen in travelers to and immigrants from endemic areas. Infection is normally initiated by the ingestion of *E. histolytica* cysts. Most cases are asymptomatic but invasion to the intestinal epithelium may cause colitis. Extraintestinal spread to the liver and other organs may follow.
Methods: All charts with the diagnosis of amebic liver abscess were retrospectively analyzed. Data of demography, travel history, clinical manifestations and outcomes were retrieved.
Results: During a ten year period (1999-2009), eleven returning travelers were diagnosed with amebic liver abscess. There were 5 women and 6 men, with a median age of 29 years (range 22–67). Eight were infected in South Asia (mainly India and Nepal), 1 in South America, and 1 in Africa (Gabon). One traveler was infected either in India or Brazil (visited both destinations). Seven were backpackers, 2 were traveling with organized tour and 2 for business. The period of time from the end of the trip to the development of clinical symptoms ranged from 1 month to a possibly up to 3 years, with a median duration of 4 months. All patients were hospitalized. Clinical symptoms before admission lasted from 5 days to 1 month. All presented with fever and right upper quadrant abdominal pain. Two patients complained of right chest discomfort and in one patient the major complaint was dyspnea. Associated gastrointestinal symptoms were present in 5 patients. Laboratory findings revealed leukocytosis and mild to moderate elevation of alkaline phosphatase. Abdominal ultrasound detected liver abscess in all patients. Serology for amebiasis (indirect hemaglutination) was positive in 6 out of 8 patients (no data available for 3 patients). Most patients had a single abscess at the right hepatic lobe, measuring 2-11cm in diameter (average 6 cm), which was drained under visualization of US or CT scan. Repeated US follow-ups demonstrated a slow resolution of the lesions, which disappeared over several months.
Conclusion: Amebic liver abscess is not a common disease among travelers despite their high probability of exposure to *E. histolytica* infection. Clinical symptoms may appear even after a time lag post travel, thus clinicians should be aware of this diagnosis. The differential diagnosis of late onset febrile infection after travel to the tropics is limited and should include amebic liver abscess. This emphasizes the need for travel history in every patient who presents with febrile disease.

Histoplasmosis is returning travelers in Israel: Experience of the Travel Medicine Service, Chaim Sheba Medical Center, Tel Hashomer

Segel, Michael J.[1], Yehudit Rozenmann[2] and Eli Schwartz[3]

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Background: *H. capsulatum var. capsulatum*, the etiological agent of "classical" or "American" histoplasmosis, is endemic to the Americas. Autochthonous histoplasmosis has never been reported in Israel, and only a handful of cases are described from the rest of the world outside of the Americas. On the other hand, classical histoplasmosis has been reported in travelers returning from the endemic areas of the Central and South America. The majority of reported cases were in the form of large outbreaks among groups of travelers.
Methods: The Travel Medicine Clinic of Sheba Medical Center is a tertiary-quaternary referral center for travel medicine. We reviewed all cases of histoplasmosis in returning travelers diagnosed in our center. Diagnosis was based on serology which was done at the CDC, Atlanta laboratory, and/or compatible radiological findings.
Results: A total of 17 cases (10 men, 7 women) of definite or probable histoplasmosis were identified. All had traveled to Latin America. (12 to C. America, 4 to S. America and 1 to both C. and S. America); none of our cases appeared to have acquired the infection in North America.Twelve patients presented with acute or subacute histoplasmosis, all confirmed by serology. The remaining 5 cases were identified incidentally, with suggestive radiological findings and history of travel to endemic areas. Three of the four patients tested for anti-histoplasma antibodies were sero-negative (exposure was more than 7 years before the blood test). Eleven subjects (65%) reported exposure to bats, in most cases in the bat cave of Lankin, in Guatemala. Our series includes 5 pairs of travel companions, but no larger groups of affected subjects.
Conclusion: The diagnosis of histoplasmosis should be considered in travelers returning from Latin America with a prolonged febrile respiratory illness. Furthermore, patients with lung nodules should be questioned regarding their travel history and histoplasmosis should be considered if the patient visited endemic areas, particularly bat-inhabited cavities. Serological testing is probably insensitive in cases in which several years have elapsed since exposure.

Laboratory tests for parasitic diseases in Israel

Marva, Esther & Tamar Grossman

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Microscopic examination is still considered the gold standard for the diagnosis of parasitic diseases. In many clinical laboratories in hospitals and in Kupot-Holim an excellent microscopic identification of parasites is performed. Microscopic

examinations using wet mount preparations are performed for the detection of protozoan trophozoites and cysts (i.e. *Entamoeba histolytica, Giardia lamblia)* and helmintic ova (*Ascaris lumbricoides)* and larvae *(Strongyloides stercoralis*). Specific concentration techniques, including flotation and sedimentation procedures are further performed for the diagnosis of parasitic diseases. However, microscopic examinations are time consuming, non-sensitive and not always reliable. For example, the identification of *E. histolytica* can be poor, confounded with false-positive results, due to misidentification of macrophages as trophozoites, PMNs as cysts and other *Entamoeba* species. In addition, the diagnosis of certain infections is not always possible by searching for the parasites in host tissues or excreta since risky invasive techniques might be necessary to locate the parasites. This is the case regarding toxoplasmosis, toxocariasis, cysticercosis and echinococcosis. Detection of antibodies can be very useful as an indication for infection with a specific parasite. A positive result in an individual with no exposure to the parasite prior to recent travel in a disease-endemic area may be interpreted as indicating recent infection. In addition to serology, there are other tests of high sensitivity which can be integrated with microscopy, such as antigen detection in stool and blood samples as well as the use of other molecular diagnosis methods. There are two main laboratories in Israel where parasitic diagnosis is available by integration of microscopy, serology, antigen detection and molecular methods: The Reference Laboratory for Parasitology in Jerusalem at the Central Laboratories of the Ministry of Health (MoH) and the Laboratory of Parasitology at Soroka University Medical Center, Beer Sheva (SOR). There are also two special diagnostic units, one responsible for the identification of toxoplasma: Reference Laboratory for Toxoplasmosis, Public Health Laboratory, Ministry of Health, Tel Aviv (Tox), and the other for the identification of Leishmaniasis: Kuvin Center, Faculty of Medicine, Hebrew University of Jerusalem (Kuv**). Serology: Amebiasis** (MoH, SOR, Elisa) , **Chagas disease** (MoH, Elisa), **Cysticercosis** (MoH, Elisa), **Echinococcosis** (MoH, SOR, Elisa; SOR: immunoblot, Arc 5, immunoelectrophoresis), **Leishmaniasis** (SOR, Kuv, Elisa), **Malaria** (SOR, IFA), **Schistosomiasis** (MoH, SOR, Elisa), **Strongyloidiasis** (MoH, SOR, Elisa), **Toxocariasis** (MoH, SOR, Elisa),**Toxoplasmosis** (SOR: IgG, IgM, competition Ab, Avidity – Elisa, Tox: Sabin Feldman, ELFA- IgM, ELFA total Ig, IgG- Avidity, ISAGA IgM); **Trichinellosis** (MoH, SOR, Elisa). **Molecular Diagnosis**: Cutaneous leishmaniasis (CL): *Leishmania major, L. tropica* and South American species, and visceral (VL) leishmaniasis, caused by *L. infantum* or *L. donovani*. (SOR and Kuv - all species by ITS1 –PCR or Real-time HRM PCR). **Malaria**: *Plasmodium falciparum, P. malariae, P. ovale, P. vivax* and soon *P. knowlesi* (MoH, Nested PCR and Real-Time PCR). **Toxoplasmosis**: Nested PCR and Real-Time PCR (Tox). **Antigen detection**: **Amebiasis:** *E. histolytica* in stool (MoH, SOR, Elisa) , **Cryptosporidiosis** and **Giardiasis –** *Cryptosporidium* and *Giardia* in stool (MoH, SOR, Elisa-immunocard). For *E. histolytica, Cryptosporidium* and *Giardia* - Real-Time PCR method will be available in MoH. **Ectoparasites** Identification of ectoparasites such as ticks, mites, lice, fleas, bedbugs, dipteran larvae causing myiasis, and other Diptera, (Entomology Laboratory, MoH).

Monday, December 28, 2009

14.00 – 15.30 **Parallel sessions (Tropical Medicine and Molecular Parasitology)**

14.00 – 15.30 **Session 8: Molecular Parasitology**
Chairpersons: Dan Zilberstein & Hagai Ginsburg

14.00 – 14.15 **Fastman,Yair & Ron Dzikowski. Switching patterns of virulence genes associated with the onset of malaria**

14.15 – 14.30 **PasternakNoa D. & Ron Dzikowski. Characterization of the nuclear pore complex and its possible role in gene expression in the malaria parasite *Plasmodium falciparum***

14.30 – 13.45 **Katz, Sophie, Oded Kushnir, Tal Lavi, Rama Siman-Tov & Serge Ankri. Insights into the up-regulation of *Entamoeba histolytica* methylated LINE binding protein (EhMLBP) expression under heat shock and its biological meaning**

13.45 – 15.00 **Inbar, Ehud, Doreen Shliselberg, Marianne Suter-Grotemeyer, Doris Rentsch & Dan Zilberstein. Molecular characterization of a proline transporter from *Leishmania donovani***

15.00 – 15.15 **Zinoviev, Alexandra, Yael Yoffe & Michal Shapira. Evolutionary diversity of the trypanosomatid cap4-binding complex – a potential drug target against trypanosomatids?**

15.15 - 15.30 **Tsygankov, Polina, Michael Kuzyk, Christoph Borchers & Dan Zilberstein. Phosphoproteomic analysis of *Leishmania donovani* development**

15.30 – 15.45 **Discussion**

15.45 – 16.00 **Closure of the Annual Meeting**

Switching patterns of virulence genes associated with the onset of malaria

Fastman, Yair and Ron Dzikowski

Department of Microbiology and Molecular Genetics, IMRIC, The Hebrew University, Jerusalem, Israel

Plasmodium falciparum is the causative agent of the deadliest form of human malaria. The primary antigenic and virulence determinant is *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) expressed by the parasite on the surface of infected red blood cells. These polymorphic proteins encoded by a multi copy gene family named *var*. Only a single *var* gene is expressed at a time while the rest of the family remains transcriptionally silent. Switching between different *var* genes is determining the antigenic, cytoadherent, and virulence properties of the infected erythrocyte. The onset of malaria symptoms is associated with release of merozoites from hepatocytic schizonts into the blood circulation and their propagation within the erythrocytes. This is exactly the time when *var* gene expression begins. In order to investigate whether specific *var* genes are associated with the onset of the disease we employed a recently developed technique that enabled us for the first time to silence the expression of the entire *var* gene family. We transfected parasites with episomes carrying multi copy active *var* promoters, thus, achieving complete silencing of endogenous *var* genes by promoter titration and effectively mimicking *var* genes expression in liver stages. We then removed the competing episomes allowing the parasites to express endogenously *var* genes, similar to early merozoites at the beginning of the disease. Using this method we were able to erase three different clonal populations expressing a subtelomeric *var* gene, a central *var* gene and the gene that is associated with pregnancy malaria known as *var2csa*. We followed their switching patterns over a period of 6 months and currently analyzing it. We hope that our analysis will unveil possible association between subset of *var* genes and the onset of the malaria.

Characterization of the nuclear pore complex and its possible role in gene expression in the malaria parasite *Plasmodium falciparum*.

Pasternak, Noa D. and Ron Dzikowski

Department of Microbiology and Molecular genetics, IMRIC, The Hebrew University, Jerusalem, Israel

Plasmodium falciparum is the causative agent of the deadliest form of human malaria. Its virulence is attributed to its ability to evade the immune system by antigenic variation. Antigenic variation is achieved by switches in the expression of the hyper-variable, multi copy *var* gene family. Silent *var* genes have been proposed to cluster at the nuclear periphery and upon activation, a single *var* gene moves out of the cluster to a *var* specific expression site located also at the nuclear periphery. We hypothesize that this transcriptionally active site might be located at the nuclear envelope with proximity to the nuclear pore complex (NPC). The NPC in all eukaryotic cells is assembled by approximately 30 proteins forming a basket like structure responsible for mediating between the nucleoplasm and the cytoplasmic environment. Recent data has revealed that besides its importance in export and import of macromolecules, the NPC is associated with transcriptionally active genes. Thus far, the NPC of *Plasmodium falciparum* was not investigated structurally or functionally. Here we show for the first time structurally defined *P.falciparum* nuclear pores using different methods of Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). In addition, we have identified four *P. falciparum* proteins that share homology with yeast NPC proteins: *pf*NUP100, *Pf*NPL4, *Pf*NUC116 and *pf*NUP2. *Pf*NPL4 was cloned and expressed fused to a *myc* epitope tag. Using these transgenic parasites we were able to describe its expression pattern and cellular localization in blood stages parasites. We are in the process of characterizing other component the NPC and its role in the gene regulation in *P. falciparum*.

Insights into the up-regulation of *Entamoeba histolytica* methylated LINE binding protein (EhMLBP) expression under heat shock and its biological meaning

Katz, Sophie, Oded Kushnir, Tal Lavi, Rama Siman-Tov and Serge Ankri

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Amebiasis is caused by the single-celled protozoan parasite *Entamoeba histolytica*, and has a worldwide distribution with substantial morbidity and mortality. EhMLBP is a nuclear protein essential for the growth and the virulence of the parasite. EhMLBP binds to methylated repetitive DNA and it constitutes with the DNA methyltransferase Ehmeth the core enzymes of the epigenetic machinery present in the parasite. Adaptation to environmental stress is a key process that allows the unicellular parasite *Entamoeba histolytica* to survive in its human host. The presence in EhMLBP of a Heat Shock Protein domain and the up-regulation of EhMLBP expression under heat shock by a mechanism that involves the binding of a heat shock transcription factor to EhMLBP promoter suggest that this protein plays an important protective role against heat shock. This hypothesis is supported by experimental data showing the better resistance of trophozoites that constitutively overepress EhMLBP to heat shock compared to control trophozoites. EhMLBP is a perinuclear protein that presents a diffuse nuclear localization following heat shock or in trophozoites that overexpress EhMLBP. What happen to the DNA binding pattern of EhMLBP in heat shocked trophozoites was unknown. We will present some experimental evidences that support a differential binding under heat shock of EhMLBP to its DNA targets. To the best of our knowledge, this work is the first report of a DNA binding protein that includes a HSP domain and whose expression can be induced by heat shock.

Molecular characterization of a proline transporter from *Leishmania donovani*

Inbar, Ehud[1], Doreen Shliselberg[1], Marianne Suter-Grotemeyer[2], Doris Rentsch[2] & Dan Zilberstein[1]

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

We have identified and functionally characterized a proline transporter (*Ld*AAP24, LinJ.10.0760) in the plasma membrane of *Leishmania donovani* promastigotes. Heterologous expression in *Saccharomyces cerevisiae* mutants indicated that LdAAP24 has low affinity (K_m=1mM), high capacity and low specificity to proline. This transporter is insensitive to external pH with a small peak of activity at pH 6.5. Polyclonal antibodies that were raised against the hydrophilic N-terminus localized LdAAP24 to the plasma membrane and flagella pocket. It is exclusively expressed in promastigotes and both LdAAP24 mRNA and protein disappeared within 2.5 hours after differentiation of promastigotes to amastigotes initiated. Knocking out *LdAAP24* from its chromosome revealed a second, high affinity and low capacity proline transporter. While LdAAP24 resembles the previously described cation-dependent system A, the second transporter is the cation-independent system B. ΔLdAAP24 lost most of its cellular pool of proline and about half that of glutamate and alanine. In addition, glutamate transport in mutant cells was about half of the glutamate transport in the Wt. Surprisingly, mutant cells doubled their cellular pool of arginine even though its rate of uptake remained unchanged. Finally, we identified and demonstrated proline transport activity of LdAAP24 orthologues in *Trypanosoma cruzi* and *T. brucei* (TbAAP24 and TcAAP24, respectively). This study facilitates the first molecular characterization of proline transport in parasitic protozoa.

Evolutionary diversity of the trypanosomatid cap4-binding complex – a potential drug target against trypanosomatids?

Zinoviev, Alexandra, Yael Yoffe and Michal Shapira

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All trypanosomatids possess a highly modified cap-4 structure at the 5' ends of their mRNAs, which is donated by the spliced leader RNA during trans-splicing. We have analyzed the cap-binding complex of *Leishmania*, in attempt to examine their roles in the different life forms of the parasite. The subunits of the trypanosomatid cap binding complex have evolved to bind the unique cap-4 structure and are therefore structurally diverged from orthologues of higher eukaryotes. We identified four eIF4E candidates in the TriTryp genome database and at least five eIF4G candidates. The evolutionary diversity of the trypanosomatid eIF4E proteins is emphasized by their inability to functionally complement the mutated yeast protein, unlike the *Drosophila* and *Arabidopsis* eIF4Es. In the absence of a functional system for in vitro translation, their functional annotation is complex and was based on binding kinetics to a synthetic cap-4 analogue, migration on sucrose gradients and the ability to generate eIF4E-eIF4G complexes. LeishIF4E-4 is the most probable basal translation initiation factor, and LeishIF4E-1 seems to be a factor that functions at mammalian-like temperatures. Analysis of their binding partners revealed the parasite eIF4G homologue, which also reflects evolutionary changes that occurred in accordance with the requirement to bind cap-4. Pull down assays shed additional light on the cap-binding complex in *Leishmania*, revealing several interesting proteins, including a novel 4E binding protein, as well as two DEAD-box proteins, functional homologues of the yeast Ded1p. The effect of temperature elevation on the cap-binding complexes is currently underway.

Phosphoproteomic analysis of *Leishmania donovani* development

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Previous LC-MS/MS based iTRAQ proteomic analysis, using our host-free system that simulates *L. donovani* differentiation, showed that most changes in protein abundance occurred at later stages of parasite intracellular development. Further studies indicated that post-translation modifications (i.e. phosphorylation, methylation, acetylation and fucosylation) also play a role in protein function and abundance changes during *L. donovani* development. Here, we aimed to determine time course changes in protein phosphorylation in order to; 1) characterize the dynamics of protein phosphorylation throughout differentiation 2) identify novel phosphorylation motifs, and 3) identify potential protein kinases and phosphatases for drug targets. In collaboration with UVic proteomic center we have devised a phosphopeptide enrichment protocol using TiO_2 that was followed by iTRAQ labeling. Currently, we have completed the first round of *L. donovani* promastigote to amastigote differentiation analysis. To date, we have identified a few hundred peptides at high confidence, most of which undergone phosphorylation during differentiation. Interestingly, a few of the changes were transient, either up or down, suggesting an important role in this process. The analysis revealed novel protein kinases and phosphatases, whose phosphorylation increased at later stages of differentiation. These and others are likely to be rational candidates for drug development.