ABSTRACTS

1. Parasite and host contributions to the pathogenesis of amebiasis

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Entamoeba histolytica is a protozoan parasite of the human intestine that causes amebic colitis and liver abscess. The pathologic hallmark of amebiasis is destruction and ulceration of the intestinal epithelium. Invasion *in vitro* is a sequential process of adherence of parasite to the host via the *E. histolytica* Gal/GalNAc lectin, adherence-dependent cytotoxicity mediated by host caspase 3 - activated apoptosis, and finally amebic ingestion of the apoptotic cell corpse. Inhibition of the Gal/GalNAc lectin (by inducible expression of dominant negative mutants in the parasite or by antibody neutralization) blocks *in vivo* virulence. Despite the robust *in vitro* virulence of *E. histolytica,* only a minority of those infected develop disease. For example our 4 year prospective study of children from an urban slum in Dhaka, Bangladesh has demonstrated that only one fifth of the 40% of children infected each year develop colitis or diarrhea. Children with disease do not significantly differ in environmental factors such as nutritional status, age, sex, duration of breast-feeding, or household income. A host genetic contribution to susceptibility is suggested by this heterogeneity in clinical outcome of infection, and by the failure of all children to become infected over the 4 years of observation despite exposure. A genetic contribution to both disease acquisition and progression is suggested by our identification of the heterozygous class II haplotype DQB1*601/DRB1*1501 significantly associated with protection from infection and disease in these children. A parasite genetic contribution to the outcome of infection is also likely. Genotyping of *E. histolytica* isolates from 111 individuals has identified 38 genotypes, with 12 predominant genotypes comprising 70% of all isolates. Genotypes 1 and 4 were significantly associated with noninvasive colonization, while genotypes 11 and 12 were entirely composed of invasive isolates. In conclusion the pathogenesis of amebiasis is influenced by differences in the genetic composition of both host and parasite. Future refinement of the genetics data from the field will enable a return to the laboratory to understand at a molecular level the dynamic interaction of host and parasite that results in disease or colonization.

2. TriTryp genome: an update

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Trypanosoma brucei, Trypanosoma cruzi and Leishmania major are unicellular, flagellated diploid protists that belong to the order Kinetoplastidae and lead either a monogenetic or a digenetic life cycle. An international effort has been made during the last decade to sequence the genomes of these organisms. A recent meeting in Seattle Biomedical Research Institute was organized to conclude this project and to present the results of these efforts. Our talk aims to present the major data on the TriTryp genomes as were described in meeting in Seattle.

3. Effect of Protein kinase A on infectivity and proliferation of *Leishmania*

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cAMP-dependent protein kinases (PKA) are involved in the differentiation, proliferation, apoptosis and stress responses of all eukaryotic cells. Almost nothing is known about the functional role of PKA in *Leishmania*. PKA exists as a tetramer containing two catalytic (C) and two regulatory (R) subunits. Binding of cAMP to the R-subunit causes its dissociation from the tetramer and activation of PKAC. PKA activity was assayed in leishmanial lysates by the migration of fluorescent Kemptide in agarose gels. *L. major* promastigotes showed higher PKA activity than amastigotes, and were studied further. Protein kinase inhibitor (PKI) specific for PKA inhibited its activity in lysates 56% (1 nM PKI), while the specific activators, 8-CPT-cAMP (14 μM) and Sp-cAMPS-AM (11 μM), increased PKA activity by almost 200%. Phosphodiesterase (PDE) inhibitors such as IBMX (2 mM) and Rolipram (0.38 mM), which prevent hydrolysis of cAMP to AMP by PDEs, also increased PKA activity by 124 and 106%, respectively. Effect of protein kinase inhibitors and activators on promastigote growth *in vitro* and infection of mouse macrophages was also monitored. Treatment of promastigotes with activators or inhibitors had opposing effect on parasite infection and survival in peritoneal macrophages over three days *in vitro*. Characterizing the role of PKA and its signal transduction pathways in *Leishmania* could serve as a basis for new drug target design. Identification of new drug targeting candidates is important especially due to increasing development of resistance by *Leishmania* to available drugs and to the lack of a vaccine.

4. Genotype characterization of *Anaplasma marginale* and *A. centrale* surface membrane proteins in concurrently infected cattle

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Anaplasma marginale (*Am*), which causes bovine anaplasmosis, is an obligate intraerythrocytic rickettsia. The disease is considered to be one of the major impediments to the livestock industry worldwide, and to reduce the losses caused by high morbidity and mortality cattle are vaccinated with live *A. centrale* (*Ac*), a naturally less virulent species. Of the five major surface proteins (MSPs 1-5) described for both *Anaplasma* species, MSP 4 and MSP 5 are encoded by a single gene copy, whereas MSP 1(α and β), MSP 2 and MSP 3 are encoded by a polymorphic gene family. It has recently been shown that in herds in which *Am* is endemic only one genotype was found in any individual bovine, an indication that the presence of one genotype excludes infection with other *Am* genotypes. In the present study we examined whether *Am* genotype exclusion takes place in *Ac* vaccinated cattle. Two 4-month-old calves were each inoculated intravenously with 10^8 live *Ac* or *Am* (Virginia strain) erythrocytes. A month later the calves were inoculated with 10^5 live infected erythrocytes of heterologous strains. The calves were monitored for clinical responses and analyzed for the genotype identity by PCR assays. Following heterologous cross-infection both calves developed clinical anaplasmosis with two rickettsemia peaks, each associated with the last species that was inoculated. The PCR assays based on *msp4* primers showed that concurrent infection was established in the cross-infected calves, with the simultaneous presence of *Am*- and *Ac*-specific amplicons of the expected sizes: 761 and 395 bp, respectively. Application of *msp1α* primers specific for *Am* but not *Ac* resulted in specific ampification of the 515-bp *Am* fragments from both calves. Positive reactivity was detected with *msp3* primers designed for simultaneous specific detection of the various species up to 3 months into the post-infection follow-up. A pen trial showed that there was neither infection nor genotype exclusion of *Am* in *Ac*-vaccinated calves. Moreover, this phenomenon was confirmed with samples obtained from field-grazing cattle 2 years after vaccination. Concurrent infection with specific genotypes pertaining to both *Anaplasma* species was detected. The results obtained may explain the high efficacy of *Ac* vaccine in providing protection against multiple *Am* strains in the field.

5. RNA structure determination of a 3' UTR translational control element in the Hsp83 gene of Leishmania

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In the absence of transcriptional regulation of RNA Pol II genes in *Leishmania*, translational regulation plays a key role in control of gene expression. Using a series of reporter constructs we defined a regulatory element in the 3' UTR of Hsp83 in Leishmania (nts 201-346), which is required for preferential translation of this protein at mammalian-like temperatures. However, this pattern of translation could be conferred onto a reporter gene only by fusion with a larger fragment of the 3' UTR that extended between sequences 1-472, suggesting that secondary RNA structures could be involved in directing preferential translation at elevated temperatures. We therefore attempted to map the RNA structure of the relevant 3' UTR region by chemical and enzymatic probing of single stranded regions. Multiple differences in

the overall RNA structure of the regulatory region were observed at different temperatures, which could possibly be related to the temperature-dependent pattern of translation. We also created a bioinformatic structure prediction program denoted RNACONS, to corroborate the experimental data. This program was based on identifying consensus secondary structures of RNA in multiple sequences with partial homology. Using this program we identified a large single stranded loop between positions 308-329 of the 3' UTR that was consistent in all mFold outputs and appeared in all available 3' UTRs of *Leishmania* Hsp83 genes. Indeed, chemical probing of this region indicated that the predicted site is found as a single stranded loop. The combination of chemical mapping with a bioinformatics-based prediction, strongly supports the possibility that preferential translation of the Hsp83 gene in *Leishmania* is controlled by a 3' UTR regulatory element that assigns a descrete secondary structure.

6. Cloning, functional characterization and motif analysis of amino acid transporter genes from *Leishmania donovani*

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The *Leishmania major* genome sequence was used to identify and clone eight putative amino acid transporter genes from *L. donovani* (AAPLds). Phylogenetic analysis of the *L. donovani* genes showed that seven (AAPLd 1-3, AAP8Ld, AAP11Ld, AAP13Ld and AAP15Ld) belong to the ATF-1 and one (AAP10Ld) to the APC super families. These genes have been expressed in various *Saccharomyces cerevisiae* mutants defective in different amino acid transporter genes. AAP3Ld mediated growth on histidine, lysine, arginine, phenylalanine and citrulline. Transport studies with *S. cerevisiae* cells expressing *AAP3Ld* indicated that *AAP3Ld* codes for a cationic amino acids transporter. Motif discovery tools (such as MEME) were employed on a training set containing AAP sequences from *L. donovani*, *L. major*, *Trypanosoma brucei*, *Plasmodium falciparum* as well as from known and characterized amino acid permease genes from all kingdoms. The results revealed two motifs that are specific to the genus *Leishmania* (L1 and L2 motifs), four to the family trypanosomatidae (T 1-4 motifs) and one common to trypanosomatidae and mammalian A1 and N transporters (N motif). Interestingly, most of these motifs are clustered in two regions of 50-60 amino acids. In addition, AAPLD genes that belong to ATF-1 display close phylogenic relationship to mammalian transporters. This work constitutes the first cloning and characterization of amino acid permease genes in *Leishmania*.

7. Alpha 2 macroglobulin (α2m) activity in rats infected with *Trypanosoma lewisi* and treated with cyclophosphamide and its influence on the malignancy of the disease

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T.lewisi is an obligatory, flagellated parasite of the rat. Despite the fact that naturally the rats overcome the disease, a lethal infection can be induced by the administration of an immunosuppressive agent, i.e. cyclophosphamide (Cy). Our previous study showed that rabbits injected with serum collected from rats infected with *T.lewisi* and treated with Cy (CyI) led to the production of a high level of anti-α2M antibodies. α2M is a 720 kDa proteinase inhibitor and an acute phase protein synthesized in rats in response to inflammation. It is involved in the resolution of inflammation by neutralizing proteases and pro-inflammatory cytokines, and is cleared from circulation through binding to α2M receptors found primarily on macrophages. The aim of the present study was therefore to determine the kinetics of α2M production and to examine its influence on the malignancy of the disease. The pathological developments in CyI rats were further characterized. α2M was purified using chromatographic and electrophoresis procedures and was further characterized by immunological methods. α2M production/removal from circulation was found to be severely affected by the parasite infection and by the mode of treatment given to the rats. In the rats infected with *T.lewisi*, α2M was first demonstrated and peaked on the 2nd day post infection (972 μg/ml) and then reduced gradually, reaching a level of 32 μg/ml on the 8th day post infection. No correlation between the pattern of disease and α2M levels was demonstrated. In the CyI rats, however, the level of α2M was gradually increased as the disease progressed, reaching a level of 890 μg/ml, on the 8th day post-infection. The highest level of α2M was demonstrated in rats injected intraperitoneally with the inflammatory compound – turpentine, either alone (Tp), or combined with *T.lewisi* (TpI). In both cases the level of α2M peaked (3000-4000 μg/ml) on the 2nd day post treatment, after which it reduced gradually, reaching a level of 32 and 570 μg/ml, on the 8th day post treatment, respectively. Injection of purified α2M into rats infected with *T.lewisi* led to increased parasitemia and malignancy of the disease. No α2M was demonstrated in the trypanosome homogenate. Severe pathological developments including pneumonia, edema and lymphocyte infiltrations were demonstrated in the CyI rats. The trypanosomes were heavily concentrated in the lungs > liver>spleen>heart>kidney. In spite of heavy parasitemia (50-70%) no parasites were demonstrated in the brain of any of the infected and treated rats. The present study suggests that increased levels of α2M in the CyI rats contribute to the malignancy of the disease. The high level of α2M presented in the serum of CyI rats seems to be mediated by Cy treatment, causing the depletion of phagocytic cells that are responsible for α2M removal from circulation . The accumulation of α2M in circulation may lead to enhanced activity of anti–inflammatory cytokines, including TGF- β, and neutralization of pro-inflammatory ones that mediate an immunosuppression and malignancy of the disease.

8. Inhibitory effect of C-Terminal Paramyosin fragments of *Schistosoma mansoni* to human complement

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A former study identified paramyosin, a muscle protein of invertebrates, on the surface of *Schistosoma mansoni* larvae and adults. This surface-bound paramyosin bound to C8 and C9 of human complement and inhibited complement activation in the terminal pathway. To determine the binding site within paramyosin, the 5'-terminal, middle and 3'-terminal parts of paramyosin cDNA were respectively cloned into pET28a expression vector. Recombinant 6×His-tagged paramyosin fragments were respectively expressed in BL21 *E. coli* and purified on a Ni-NTA column. Western blotting with monoclonal anti-6×His antibody showed that the C-terminal part of paramyosin (PmyC) bound to human C9. PmyC was fragmented into three parts (PmyCN, PmyCM and PmyCC) using recombinant techniques as above. Binding assays demonstrated that only PmyCC (from ^{744}Asp to ^{866}Met) of the three fragments bound to human C8 and C9. Functional analysis showed that PmyCC inhibited Zn^{2+}-induced C9 polymerization, hemolysis of rabbit erythrocytes and of antibody-sensitized sheep erythrocytes by human complement. Also, PmyCC inhibited the *in vitro* killing of trypsin-sensitized schistosomula of *S. mansoni* by human complement. Antibodies from mouse sera immunized with recombinant paramyosin bound to PmyCC, indicating that the binding site of paramyosin is predominantly located in the region between ^{744}Asp and ^{866}Met of this molecule. Taken together, these novel findings suggest that PmyCC could provide an appropriate target for vaccine design against schistosomiasis on the one hand, and as a therapeutic complement inhibitor in cases of human complement-induced diseases.

9. *Neospora caninum*: an emerging abortifacient pathogen in cattle

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Bovine neosporosis is caused by the apicomplexan protozoon *Neospora caninum*, which is known as an important pathogen that causes abortion in dairy and beef cattle worldwide, including Israel. Transplacental transmission is the major and effective route of transmission *of N. caninum* in cattle. Infection of adult cattle with Neospora parasites is not accompanied by clinical signs, therefore the diagnosis of neosporosis is based mostly on the detection of specific antibodies in serum or in fetal fluids. The immunofluorescence assay (IFA) is currently used for detection of specific antibodies. Titers of 1:80 or higher in fetal fluid or of 1:400 or higher in an aborting cow have been found to be significant indications that an abortion could be caused by *Neospora caninum*. In the present study, the nested PCR (nPCR) assay was applied for the detection of parasites in the brains of experimentally infected laboratory animals (*Meriones tristrami*), in order to determine the sensitivity of the assay, and also in bovine fetuses, as an additional tool for the definitive and reliable diagnosis of neosporosis in aborting cattle. With the primers designed for nPCR the sensitivity obtained was 5×10^2 parasites. In the infected gerbils, which developed positive IFA

titers up to 1:4096, Neospora, DNA was detected in all infected animals up to 8 months after inoculation. Parasites were isolated by infection of a cell culture between 5 and 13 days after seeding of the brain material. A total of 98 bovine fetus samples that were obtained from naturally occurring abortions in dairy cattle were tested by PCR and serology, and 30% of the fetuses and 30% of dam sera were found PCR and serologically positive, respectively. Fifty-seven samples were found negative by both assays. In 21 samples there was a discrepancy between the results obtained by PCR and by serology: 11 samples that were positive by PCR were found negative by fetus serology, and the remaining 10 samples were negative for parasitic DNA, but showed specific antibodies at titers from 1:160 to 1:1280. According to these results it appears that *Neospora caninum* is a widespread pathogen in Israeli dairy cattle, and that it plays an important role in causing abortions. Application of an integrated diagnosis based on serology, PCR, and isolation of parasites in fetal tissues by inoculation into laboratory animals or into tissue cultures will facilitate the reliable assessment of Neospora-related abortions in cattle.

10. Incidence of *Trichinella spiralis* infection in wild carnivores

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Samples of diaphragms from 122 red foxes and 66 golden jackals were examined for *Trichinella spiralis* larvae by trichinoscopy. The samples were collected from animals submitted for rabies diagnosis to the Kimron Veterinary Institute during the last year. *Trichinella* larvae were found in 4 (3.3%) foxes and 22 (33.3%) jackals with an average intensity of 43 lpg (larvae per gram) in foxes and 10.1 lpg in jackals. *Trichinella* infected animals were detected only in the northern parts of Israel and Jerusalem mountains with similar, high infection rates: 7 out of 17 (41.2%) jackals from the Golan Heights, 3 out of 12 (25%) jackals from the Upper Galilee, 8 out 21 (38.1%) jackals and 2 out of 7 (28.6%) foxes from the Carmel, 4 out of 7 (57.1%) jackals and 2 out of 6 (33.3%) foxes from the Jerusalem mountains. On the other hand, all 74 foxes and 8 jackals from a central area, as well as 27 foxes and 11 jackals from southern Israel, were found negative for infection. The preliminary data of high prevalence of *Trichinella* infection in wild carnivores in the northern mountain areas of Israel confirm the findings of high prevalence of trichinosis in wild boars on the Golan Heights (9.2%) and Western Galilee (8%) and the absence of *Trichinella* infection in wild boars from the central costal plain and southern Israel.

11. Studies on blood parasites of birds in migration via Israel

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Israel is located on the main route of bird migration between West Eurasia and Africa. With over 500 million birds (of over 200 species) passing through, Israel inevitably becomes committed to world community to safe guard this migration phenomenon. In order to consolidate and activate conservation policies it is imperative to develop deep insight of the biological and ecological factors, which are involved in the migration process. Many of the migrating birds carry blood parasites. These malarial parasites, of the genera *Plasmodium* and *Haemoproteus* can potentially induce anemia and compromise oxygen transport to tissues. *Leucocytozoon* by congesting blood vessels was shown to congest visceral circulation. Optimal oxygen supply is critical to birds in migration. Adequate energy resources are another critical demand in migration. Limited energy resources compel birds to trade-off in allocating their energy, to compromise between energy needs (and protein resource) for migration and requirements for activation of immune defenses. For many birds, spring migration coincides with stimulation of the reproductive system. The reproductive hormones also activate relapse of malarial parasites, which further aggravate the homeostasis of the migrating bird. Blood of 749 migrating birds caught for ringing in the Jordan valley during spring and autumn 2002-2003, were examined for parasites, and studies are in progress since 2004, in Eilat (on the north end of gulf of Aqaba), where over 1000 birds have been examined. Data from the birds caught in the Jordan valley demonstrate fairly high consistency in prevalence from one year to the next, and higher infection prevalence during spring migration. In addition to parasite species already described from European birds, new ones have been detected, mainly in southern migrants.

12. Malaria prophylaxis and treatment-New advances

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13. The epidemiology of tick-borne relapsing fever in Israel

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Tick-borne relapsing fever (TBRF), commonly called in Israel 'Cave Fever' is characterized by recurring episodes of fever and nonspecific symptoms. The illness is caused by infection with *Borrelia* species that have the genetic ability to vary their surface antigens extensively, leading to repeated stimulation of the immune system by each new antigen and a febrile response in the patient. The TBRF *Borrelia* is transmitted to humans by exposure to the bite of an infected *Ornithodoros* tick. Relapsing fever is a reportable disease in Israel, both in the civilian and the military population. The demographic, clinical and geographic information from civilian and military reports during the years 1971 through 2003 was evaluated. A total of 600 cases were reported during these years, 277 (46%) of them in civilians, and 323 (54%)

in military personnel. In civilians, the incidence has declined from an average of 0.35-cases/100,000 population/year in the years 1975-1985 to an average of 0.11-cases/100,000 population/year in the years 1986-2002 ($p<0.001$). The incidence of TBRF in the military during the years 1983-2002 has been relatively constant, and there has even been a slight increase in the incidence of TBRF during the last decade, with an average of 5.9-cases/100,000 population/year in the years 1983-1991, compared with 6.5-cases/100,000 population/year in the years 1992-2002. Altogether, exposure in caves was reported in only 64% of cases. TBRF continues to be endemic in Israel, and still poses a significant hazard for soldiers and travelers, even without history of caves exposure. Although there has been a significant reduction in the incidence of TBRF in civilians, the incidence in soldiers has not declined and probably reflects more accurately the existence of the pathogen in Israel. Insufficient information is available on the habits of the *Ornithodoros tholozani* tick and its reservoirs, which is essential in order to further reduce the incidence of TBRF. In addition accurate mapping the locations infested by the *Ornithodoros* ticks is needed.

14. Post exposure prophylaxis with doxycycline for the prevention of relapsing fever

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Tick borne relapsing fever (TBRF) is an acute febrile disease characterized by a pattern of remission and relapse and caused by spirochetes of the genus Borrelia. Evidence is lacking on the safety and effectiveness of post exposure prophylactic treatment for TBRF. In this double blind, placebo controlled trial healthy volunteers with tick bites and close contacts with no bite signs were randomly assigned to receive doxycycline (200 mg for first day and 100mg/d for 4 days) or placebo. Blood smears were examined for Borrelia at inclusion and during fever rise. Serology for Lyme disease cross-reactivity and PCR for Borrelia GlpQ gene were examined. Cases of TBRF were defined as subjects having fever and a positive smear. 582 subjects in 17 cohorts at risk of exposure to Borrelia were screened, 125 were eligible for recruitment, 81 (13.9%) with tick-bite signs and 44 (7.6%) contacts. 93 (73.8%) volunteered, 51 with bite signs and 42 contacts. Volunteers were treated approximately 2 days after they were bitten. There were no significant adverse effects to treatment. Ten smear-proven cases of TBRF were identified and all belonged to the placebo group conferring a 100% efficacy of treatment. All positive cases had identifiable tick-bite marks. PCR was negative during incubation period but positive for all sick individuals during fever rise. Prophylactic treatment with doxycycline is safe and efficacious in preventing TBRF after suspected exposure in a high-risk environment. PCR for Borrelia GlpQ may help the diagnosis of acute disease but not to detection of exposure.

15. Microbiological diagnosis of relapsing fever Borrelia

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Relapsing fever (RF) in Israel is considered to be caused by the spirochete *Borrelia persica* and transmitted to humans by *Ornithodoros tholozani*. Between 1980-2002, 184 cases (i.e. an average of 8 cases per year) were reported to the Ministry of Health. All over the world, 29 species of Borrelia were described. They are the etiologic agents of two distinct diseases: Lyme borreliosis (LB) and RF. These two diseases are very different in many aspects: geographical distribution, vectors, epidemiology, pathogenicity, microbiological diagnosis, etc. Classically, the microbiological diagnosis of RF is based on thick and/or thin blood smears that need to be sampled at the time of fever peak. They are time consuming techniques that require experimented technicians and they also lack sensitivity. Other methods like dark field microscopy or quantitative buffy coat are less used. Contrary to LB strains, most RF strains required animal inoculations procedures. But recently, some species were successfully cultured on modified BSKII medium. Serology is considered of low diagnostic value because of the antigenic variation encountered by the bacterium. DNA extracted from ticks and blood samples were amplified using PCR methods. The advantages of PCR methods are high sensitivity and the ability to identify the exact infective species via sequencing. The *fla, 16s rRNA* and *GlpQ* genes were targeted. By sequencing we have identified the same Borrelia strain from DNA extracts of *Ornithodoros tholozani* ticks and patient's blood. We found 80% homology between the Israel RF *Borrelia fla* (that is probably *B. persica*) and other RF species *fla* sequences. Taxonomic analysis showed that sequences of the Israel RF Borrelia clustered in a separate group (Middle East RF species) from the American and the African RF species. This is the first report of molecular characterization of the causative agent of relapsing fever in the Middle East.

16. Cave types of Israel and their possible relationship with the spatial distribution of *Ornithodoros tholozani*

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Israel displays a gradient of cave features from the intensive karstification of Lebanon in the north to practically no karst caves in Eilat region at the southern Negev desert. This is attributed mainly to the climatological gradient from alpine-Mediterranean climate in the Lebanon - Hermon mountains in the north, with precipitation >1000 mm/year, to the extremely arid southern Negev, with <50 mm/year. Another factor is the southward decrease in carbonates/clastics ratio of the phanerozoic stratigraphic section. Most caves developed horizontally in limestone. Today these caves are either dry or experience vadose dripwater. Some of them have been isolated from the surface until opened by recent construction activity. Vertical caves experience some water flow and active dissolution during the rainy season. The unique rock salt karst of Mount Sedom exhibits the largest salt caves known in the world. Israel is

especially rich in man made caves sustaining abundant fauna. Preliminary observations indicate that the tick *Ornithodoros tholozani* does not appear in : (1) salt caves; (2) vertical caves; (3) wet, muddy caves; (4) Caves which have been isolated from the surface, and opened only recently; (5) caves with no fauna; (6) sea caves. Other factors controlling the tick's spatial distribution need to be investigated.

17. The biology of the relapsing fever tick *Ornithodoros tholozani*

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The "cave" tick, *Ornithodoros tholozani*, belongs to the soft-tick family Argasidae. Biological studies in Israel showed that this tick can be found in caves and archaeological sites in Galilee Mountains, Mount Carmel, Samaria and the Judean hills, coastal plain, Jordan Valley and the Negev. Rodents, porcupines, badgers, goats, sheep, cattle and humans act as hosts for this parasite. Within limestone caves and archaeological ruins, the ticks are found in shaded or dark corners, in crevices of walls (up to 1 m), in dry manure and under stones. They avoid soil, which is too wet. Mature ticks are concentrated in wall crevices, while nymphs are more frequently found in the soil near walls. In sandstone caves in the coastal plain, ticks are located in the center of caves in the top layer of sand. When a host enters the cave, larvae climb on him within seconds, followed by the younger nymphs and adults. The temperatures in these caves vary from 16–25^{o}C and the RH from 40–60%. Epidemiological studies in Israel of the areas in which humans could have been infested show that these ticks also exist in deserted or inhabited houses (rural houses in villages), bunkers, military tunnels, crevices in rocks and along the slopes of wadis. Accordingly, there is a discrepancy between the places where such ticks were found and areas in which people were infested with ticks and acquired the disease. Therefore, there is a need to examine these potential biotopes of ticks, and the environmental conditions in which they are able to exist. The study of additional biotopes would also have an importance for public health.

18. Parasitic diseases among HIV seropositive Israeli patients

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Parasitic pathogens, especially helminths and HIV are common co-infections since both pathogens are hyperendemic in the same geographical regions. It is estimated that more than 30,000,000 people are co-infected with HIV and a parasite, most of them live in Sub-Saharan Africa, Southeast Asia, Ukraine and Russia, and Middle and South America. The interaction between HIV infection and helminthic infections is complicated and in some studies it was found that the complex evolutional changes

resulting from long standing helminthic infestations may have created an opportunity for more rapid infection with HIV as well as quicker progression to AIDS. On the other hand, the diminution of the immune system may increase the risk of severe overwhelming parasitic infection, e.g. hyperstrongyloidiasis. Israel is a melting pot for people immigrating to it from all over the world, so it does not surprise that more than third of the HIV infected patients carry also a parasite. The most common parasites reported in Israeli HIV patients as causative agents for opportunistic infections are toxoplasma, intestinal parasites and leishmania. Among HIV positive immigrants from other regions of the world other parasites, especially helminths like *Necator americanus, Schistosoma mansoni, Ascaris lumbricoides, Hymenolepsis nana, Trichuris trichiura* and *Strongyloides stercoralis,* were found but the clinical significance of them is not clear yet. The study of the interaction between parasitic infection and HIV among Israeli patients continues.

19. Schistosomiasis in returning Israeli travelers

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Increased traveling, especially to the tropics, has changed the spectrum of infectious diseases seen in Israel. During the last decade, *Schistosoma* infection has emerged from the history books and become relevant to the Israeli physicians who take care of returning travelers. Our study aimed to summarize the epidemiology and clinical spectrum of schistosomiasis in Israeli travelers. During the years 1994-2003, 120 patients were diagnosed with schistosomasis in Israel, according to clinical symptoms and results of biological tests (serology, direct microscope identification of eggs in urine or stool, tissue biopsy). Data were collected from the Center of tropical diseases in the Sheba medical center, Tel-Hashomer, from the Central laboratory of the Ministry of health, Jerusalem, and the Center for Disease Control (CDC), Atlanta GA, USA. Eighty-six patients (72%) were males and the mean age was 27 years (13-54). All of the patients were infected in the sub-Saharan Africa. The two major sources of infection were Lake Malawi (65% of the patients), and participating in rafting expeditions on the Omo river in Ethiopia (23%). The mean duration of stay near the infected water source was 3 weeks (0.1-28wks). The diagnosis was made by detecting specific antibodies in 91% (110) of patients, direct microscope identification of eggs in urine 34% (24pt), stool 5% (4pt) and tissue biopsy 4% (3pt). *Schistosoma haematobium* was detected in 36%, a similar percentage 35% was identified as *Schistosoma mansoni*, and the rest of the patients did not have definitive species identification. Detailed clinical data was gathered for 72 patients: Acute schistosomiasis occurred in 68% (49 patients): fever 52% (38), cough and shortness of breath 36% (26), and urticaria 19% (8). These signs and symptoms appeared at average of 2.5 weeks after the exposure (1-16 wks). The chronic manifestations of schistosomiasis, such as: dysuria, hematuria, hematospermia, were observed in 28% (20 patients). A quarter of the patients (23%) were hospitalized for treatment and diagnosis, the majority were outpatients. The mean time from onset of clinical symptoms until diagnosis and treatment was 3 months, and in some cases was extended to two years. One case was diagnosed only ten years after the infection.

In conclusion, most cases of schistosomiasis in travelers are presenting in the early stage of the disease in which diagnosis by eggs detection is usually negative, and

Praziquantel treatment is less effective. Thus, new diagnostic methods, new drugs affecting the parasite at this stage are urgently needed. In addition a better acquaintance of schistosomiasis among the medical community should be encouraged.

20. Effect of repeated chemotherapeutic treatment of hydatidosis patients on specific anti-echinococcal antibody avidity/activity

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Cystic echinococcosis (Hydatidosis) (CE) is a severe, zoonotic parasitic disease caused by the cestode *Echinococcus granulosus*. Clinical evaluation of the outcome of treatment/surgery is not always possible due to the presence of high level of antibody activity over many years post successful treatment. The present study is aimed at determining a). the effect of a repeated treatment given at various times post treatment/surgery on the specific anti-echinococcal antibody activity and b). whether repeated treatment should be considered in these cases. Six patients, aged 23-62 years, suffering from CE underwent surgery for the removal of echinococcal cysts. All patients were treated with albendazole prior to and following treatment. Ultrasound and roentgenography either alone or combined with computed axial tomography (CAT) that were performed at various times after surgery, did not reveal any cyst in the patients examined, except for one suffering from hydatidosis of the spine. Both, ELISA, immunoelectrophoresis and immunoblot were used to determine specific antibody (IgG; IgG4, IgE) activities. Prior to the first treatment/surgery, anti-echinococcal IgG, IgG4 activities to echinococcal antigens were observed in all patients, while IgE was presented only in 5 out of the six patients examined. Total elimination of IgG and IgG4 antibodies was not achieved in any of the patients studied, while IgE was reduced to zero in 2 patients, 5 and 10 years post treatment. The 1st treatment/surgery was followed by a highly elevated IgE in 2 patients, in one of them it was further combined with an apparent decrease in IgG activity. A repeated treatment with albendazole given 1.5-8 years after the first treatment/surgery was followed by either a moderate or a highly reduced IgE activity in 2 patients and a slight increase in IgG4 in an additional patient. A 3ed course of treatment, given 2-3 yrs after the 2nd treatment barely affected the antibody activity. IgG activity was always higher than that of IgG4 over the study period. A different pattern was observed with IgE activity, showing an initial IgG/IgE ratio of 2.0 – 4.4 in 4 patients, a converted ratio (IgE/IgG) of 2.5 in one patient and no IgE activity in an additional patient. Afterward, the IgG/IgE ratio was >1.0 in 3 patients and <1.0 in additional 2 patients. In the later 2 patients a conversion of IgG/IgE ratio from <1.0 to >1.0 was observed 1 and 1.5 years after surgery/treatment. An avidity test performed during the 11 years follow-up period, using both , 1-3M urea and 0.05-0.3M potassium thiocyanate, did not show any change in the affinity of both IgG and IgG4 to echinococcal antigens. IgG4 showed lower affinity to both antigen A (20 kDa) and antigen B (8,16,24 kDa) compared to IgG , as determined by immunoblot. The present study suggests that a). anti-echinoccocal antibody activity may remain high

many years after successful cyst removal. b) Total IgG, rather than IgG4 or IgE response to HCF, has a diagnostic value in the diagnosis of echinococcal disease. c) Follow-up of IgG + IgG4 + IgE is preferable to IgG alone in determining treatment results. d) Total IgG titers may remain positive for many years following intervention and therefore there is no justification for any secondary treatment, if imaging techniques do not reveal any cysts. The present study was supported by INCO.DC grant No. 98/99.

21. A familial outbreak of opisthorchiasis due to consumption of raw fish illegally imported from Siberia

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Liver fluke infection caused by trematodes belonging to the family Opisthorchiidae remains a major public health problem in many parts of the Far East, Southeast Asia and Eastern Europe. However, with the growing volume of international travel and populations migration the infection is increasingly diagnosed in non-endemic countries, particularly in North America, among immigrants from Southeast Asia. We describe an outbreak of acute opisthorchiasis in a family who was infected in a non-endemic country following consumption of raw carp fish illegally imported from Nizhnevartovsk, situated in a highly endemic area in Siberia. Opisthorchis/Clonorchis eggs were detected in stool samples of 4 out of 5 persons who ate from the fish; two patients developed significant acute illness consisting of fever, gastrointestinal symptoms, jaundice, marked leukocytosis with important eosinophilia, and elevation of liver enzymes. Response to praziquantel was prompt. *Opisthorchis felineus* infection is the most prevalent food-borne liver fluke infection of man in Russia, Ukraine and Kazakhstan. Infestation usually follows consumption of raw, slightly salted and frozen fish ("stroganina"). The parasite is endemic in an area that covers nearly all the territory of the Russian Federation with the exception of the northern parts of Siberia and the far-eastern regions. The largest endemic area is in western Siberia, namely the Ob and Irtysh River valleys and their tributaries. *Clonorchis sinensis* infection is endemic in the Amur River valleys and Khabarovsk territory situated in the very east of the Russian Federation. With the growing numbers of the former USSR citizens immigrating to non-endemic countries, western physicians are called for increased awareness regarding Opisthorchis-associated pathology in this population.

22. Immunomodulation as treatment for malaria

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The continuous effort invested in the discovery of drugs against malaria is directed towards the identification of new targets in the parasite. These efforts are incomplete because many aspects of the disease are caused by host immunological responses. The immune responses to *Plasmodia* and anti-malarial drugs have been extensively studied as separate subjects, but little is known about their combined effects. Chemotherapy may alleviate malaria by reducing the parasite load but also by changing the exposed antigens. This in turn could affect host pathology by altering the cytokine cascade. In addition, anti-plasmodial treatment may reduce toxic parasite products accumulated by phagocytosis of infected cells which hamper "positive" macrophage activities. The significance of certain drug effects is discussed. Chloroquine is not effective against many *P. falciparum* strains and its antiparasitic effect is reduced even more because it decreases the TNF level. In contrast, treatment with iron chelators increases the TNF level and this correlates with reduction of parasitemia, in a mouse model. However, if high levels of TNF are produced, ICAM-1 expression is up-regulated, thereby, enhancing sequestration of parasitized erythrocytes and the consequent local pathology. Therefore, the TNF level must be balanced so that only the parasites are affected and there is no pathological damage. Because immunomodulation may affect the antioxidant status of the infected host and consequently that of the parasite, it should be considered when using drugs whose effect depend on the redox status. Thus, oligonucleotides containing an immunostimulatory sequence (CpG) enhance effector functions which lead to oxidant stress and reduction in the level of glutathione (GSH) – this should lead to enhanced antimalarial action of chloroquine and amodiaquine. In contrast, while N-acetylcysteine (NAC) alleviates the symptoms of cerebral malaria, it also increases the GSH level, thereby antagonizing the effect of these drugs. The severity of malaria depends on the immune status of the patient. In view of the dual sword effects of the immune responses (protective or immunopathological), an adjuvant therapy that will tip the balance towards recovery is needed. Drugs should be designed to suit the specific sympthoms and their combinations should be carefully examined in view of their effects on the immune functions.

23. Early cellular and humoral responses in cutaneous leishmaniasis patients staying in endemic region in southern Israel

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Limited data are available regarding the early immunological events associated with human cutaneous leishmaniasis (CL). This information is particularly important to address the effect of treatment on the development of a protective immune response. A novel flow-cytometry-based technique has been used to determine the cellular immune response (CIM) of peripheral blood mononuclear cells (PBMC) in response to freeze-thaw *L.major* soluble antigen (FTS). Thirty five persons with CL lesions and 32 without lesions (NL) that were staying in Ketzioth, an endemic region for CL in Southern Israel, were examined. PBMC were labeled by carboxyfluorescein diacetate fluorescent dye and exposed to FTS for six days. CIM has been evaluated according to a decrement in green fluorescence (proliferation, PF) and changes in light scatter

(blast morphology). Cases with PF fraction of <0.15 and lacking blasts were considered negative. Those with PF fraction of 0.15–0.30, 0.31-0.64 and ≥ 0.65 and containing blasts were considered weak positive (+), moderate positive (++) and strong positive (+++), respectively. All CL patients (35) were examined 1 - 3 months after the first appearance of the disease. In 15 of this group the disease was confirmed parasitologically (smear + culture), and in the remaining 20 cases, the disease was only clinically confirmed. Amongst the 35 CL cases, 51.42% (18/35) displayed a BT [7 (20%) +, 8 (22.8%) ++ and 3 (8.6%) +++]. Amongst the 15 parasitologically confirmed cases, 10 (66.7%) displayed a BT [4 (26.7%) +, one (20%) ++ and 3 (20%) +++]. Amongst the 20 clinically confirmed cases 8 (40%) displayed a BT [3 (15%) + and 5 (25%) ++]. In the 32 NL group only 3 (9.4%) displayed a BT [2 (6.25%) + and 1 (3.1%) ++]. The extent of cellular reactions was only partially (33.3%) correlated with the humoral reactions as was determined by ELISA. In the CL group (35), 34.3% (12/35) displayed both cellular and humoral immune response, while 14.3% (5/35) and 28.6% (10/35) displayed only cellular or humoral reactions, respectively. Amongst the parasitologically confirmed cases (15/35), 26.7% (4/15) displayed both cellular and humoral immune response, while 40% (6/15) and 26.7% (4/15) displayed only cellular or humoral reactions, respectively. No humoral response was detected in the NL group (32) and only 3.1% (1/32) of them displayed cellular response. Out data clearly indicate that CIM develops very early after the appearance of CL lesion, and probably immediately after initial infection. We suggest that the novel flow cytometry approach described in this study may serve as a useful tool for the evaluation of CIM, prior and post treatment. It can further be used for the elucidation of the responding lymphocytes (CD profile of proliferating cells) and the reaction type (Th1 or Th2 cells) by measuring the intra-cellular cytokines.

24. Pentostam resistance in an infantile visceral leishmaniasis case from Hebron District

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Leishmania infantum causes Infantile Visceral Leishmaniasis (IVL) in the Mediterranean region. Here we describe the case of a 4 years old male with a history of high fever, acute anemia, leukopenia and hepatosplenomegaly for over six months. A bone marrow smear was stained and examined, and leishmanial antibodies were tested by an enzyme-linked immunosorbent assay (ELISA) in two different laboratories. The patient was treated for three weeks with 20 mg/kg/day Pentostam with no response or indicator of improvement, later this drug was replaced with intravenous Amphotericin B (Ambisome) 0.25 mg/kg/day. Histology examinations showed no amastigote-like bodies. Acute pancytopenia was observed, but ELISA was highly positive for IVL in the two different laboratories. The patient responded well to intravenous Amphotericin B and blood indicators improved a few weeks after beginning the Amphotericin B course. Regional clinicians must keep in mind all infectious agents as differentials for abdominal enlargement and fever of unknown origin. Microscopic examination of bone marrow smears is of limited sensitivity and

cases of IVL are frequently missed. Therefore, a simple ELISA for IVL may give a clue to the diagnosis in these situations. While pentostam resistance to *L. infantum* infections in our area is extremely rare, treatment failure is not indicator for misdiagnoses. In these cases use of alternative therapies should be considered.

25. Anti-leishmanial activity of the Dermaseptin derivatives: A systematic approach using axenic amastigotes and promastigotes of *Leishmania donovani* and infected THP-1 macrophages

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Leishmaniasis causes significant mortality and morbidity worldwide. Toxicity, cost, drug resistance and parenteral administration all make treatment difficult. Over the last two decades small cationic peptides (SCPs), important components of the innate defenses of most species, have been shown to be active against a large spectrum of microorganisms including parasitic protozoa. The aim of this study was to systematically investigate the Dermaseptin (DS) SCP family's anti-*L. donovani* activity by changing hydrophobicity, positive charge and SCP length, using axenic amastigotes and promastigotes as well as infected THP-1 macrophages. Eleven DS derivatives were blindly supplied by Prof. Mor's laboratory. *L. donovani* (LdS1) axenic amastigotes and promastigotes were exposed to different DS derivative concentrations in 96 well plates. After a short incubation (1.5 – 3 hr), parasite viability and growth were assessed by tritiated thymidine incorporation. A potent, short peptide (K_4S4(1-15)a) was selected in order to visually assess the DS mechanism of action. Fluorescent and confocal microscopy was used. Green fluorescent protein-containing promastigotes and axenic amastigotes were exposed to rhodamine-labeled K_4S4(1-15)a. Propidium iodide served as a marker of membrane integrity. DS derivative toxicity to THP-1 macrophages was assessed in 2 ways: XTT reaction kit, and incorporation of tritiated proline. THP-1 macrophages infected with *L. donovani* were exposed to K_4S4(1-15)a and stained with Diff Quick (Dade-Behring) in order to assess drug selectivity (parasite vs macrophage toxicity). Various DS derivatives are cytolytic at very low concentrations (2- 40 mcg/ml). Promastigotes are 3 – 4 times more susceptible to DSs when compared to amastigotes (regardless of their hydrophobicity, charge or length). DS hydrophobicity is independently associated with SCP anti-leishmanial activity. Using fluorescent microscopy at sub-lytic concentrations, K_4S4(1-15)a crosses the parasite plasma membrane without causing significant membrane damage, suggesting an intracellular anti-leishmanial affect. The DS derivatives investigated are toxic to THP-1 macrophages. Significant cytolytic activity at very low concentrations and evidence of intracellular penetrating capability suggest that SCPs, including the DS family, may be potential agents for the treatment of cutaneous and visceral leishmaniasis. Flexible, easy and targeted peptide synthesis may provide the key for increasing SCP activity and diminishing SCP effects on host cells, both in vitro and in vivo.

26. A trichinellosis outbreak : epidemiology, serology, parasitology and public health

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Trichinellosis is a zoonotic disease caused by the nematode *Trichinella*. Up until 1992, only two outbreaks were reported in Israel. From 1998 to 2002, 6 identical Trichinellosis outbreaks occurred involving 120 Thai workers employed in agriculture. They resulted from consumption of raw meat from wild boars slaughtered by hunters in the Upper Galilee, and distributed without veterinary supervision. One outbreak took place in the Ashkelon district among a group of 47 Thai workers. Twenty six of them were symptomatic (55%), 14 asymptomatic with at least one positive serology (30%) and 7 healthy workers whose serology remained negative during two months of study. The commonest symptoms were muscle soreness and edema of the upper eyelids. Sera of the 47 workers who all participated in the meal were tested for the presence of anti-*Trichinella* antibodies. The first serum was taken at the onset of the clinical symptoms. Forty of the workers (85%) had at least one positive serology. A sample of intercostal muscles from the implicated pork meat was found to contain encysted *Trichinella spiralis* larvae. These outbreaks show the need for increased awareness of the population to this public health risk both in the health care system and among consumers. Outreach to migrant workers, who may not have access to the usual channels of information, must be part of the standard public health approach in the prevention of trichinellosis.

27. Mechanism of MSP2 antigenic variation in cattle persistently infected with *Anaplasma centrale*

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Anaplasma, a member of the ehrlichial genogroup II, is an intra-erythrocytic pathogen, containing a circular genome with estimated size of 1.2 to 1.6 Mb. Two *Anaplasma* spp. infect cattle: *A. marginale* and *A. centrale.* A live strain of *A. centrale* that causes only a mild form of anaplasmosis is used for vaccine production in Israel. Major surface protein 2 (MSP2) of *A. centrale* is encoded by a polymorphic multigene family, approximately 36 kDa in size. Antigenic variation of *Anaplasma* MSP2 occurs during persistent infection and enables the rickettsiae to evade the pre-existing immune response. MSP2 is composed of conserved amino- and carboxy-terminal regions flanking a central hypervariable region, characterized by substitutions, insertions and deletions. *A. centrale* MSP2 is expressed from the operon, which has four open reading frames (ORFs) containing *msp2* at the 3' terminus. The operon structure was shown to be conserved among *A. marginale* and *A. centrale* strains. The operon is the only expression site of the full-length *msp2* transcripts. The sequences of the three upstream *orf*s are conserved; the

polymorphism is found within the *msp2* coding region in the hypervariable region. Seven *msp2* pseudogenes were identified in the *A. centrale* genome. The pseudogene copies of *msp2* in the genome are truncated: they contain a central hypervariable region flanked by short portions of the 5' and 3' conserved regions. We have shown that in *A. centrale* MSP2 variants are generated by two mechanisms: recombination of the whole pseudogene into the single *msp2* expression site, and recombination of small segments of pseudogenes into the expression site by segmental gene conversion. *A. centrale* must infect vaccinates in order to confer immunity; thus generation of *A. centrale* MSP2 variants and induction of the immune responses to conserved B and T cells epitopes enables control of the high-level rickettsemia in response to *A. marginale* challenge. The efficacy of the *A. centrale* vaccine suggests that knowledge of the strategies used by rickettsia to mimic induced immune responses will provide the key to development of an effective, non-blood-based vaccine.

28. Cap-binding activity of an eIF4E homolog from Leishmania

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All eukaryotic mRNAs posses a 5' cap that is composed of m^7GpppN (where N is any nucleotide). The cap structure is recognized by members of a family of cap-binding proteins that participate in various processes, such as RNA transport and stabilization, as well as in assembly of the translation initiation complex. The 5' cap of trypanosomatids is complex; in addition to the consensus 7-methyl guanosine, it includes unique modifications on the first four transcribed nucleotides, and is thus denoted cap-4. Here we analyze a cap-binding protein of Leishmania, in an attempt to understand the structural features that promote its binding to this unusual cap structure. We show that LeishIF4E-1, a homologue of eIF4E, contains the conserved cap-binding pocket, similar to its mouse counterpart. The mouse eIF4E has a higher K_{as} for all cap analogues tested, as compared to LeishIF4E-1. However, whereas the mouse eIF4E shows a 5 fold higher affinity for m^7GTP than for a chemically synthesized cap-4 structure, LeishIF4E-1 shows similar affinities for both ligands. A sequence alignment shows that LeishIF4E-1 lacks the region that parallels the C-terminus in the murine eIF4E. Truncation of this region in the mouse protein reduces the difference that is observed between its binding to m^7GTP and cap-4, prior to this deletion. Based on our experimental data, we hypothesize that variations in the structure of LeishIF4E-1, possibly also the absence of a region that is homologous to the C-terminus of the mouse protein, promote its ability to interact with the cap-4 structure. LeishIF4E-1 is distributed in the cytoplasm, but its function is not clear yet, since it cannot substitute the mammalian eIF4E in a rabbit reticulocyte *in vitro* translation system.