the appendages, suggesting that ciliates can more easily colonize less active body parts.

Cutaneous Leishmaniasis in Northern Afghanistan. M.L. AMIRI\*, M.Z. AAMOON\*\* and N. JALILI\*\*\*, \*Institute of Malaria and Parasitic diseases, Mazar-e-Sharif, Afghanistan, \*\*Army Hospital, Mazar-e-Sharif, Afghanistan, \*\*\*Institute of Parasitology, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic.

From April 1989–December 1999, 21405 positive cases of cutaneous leishmaniasis, were registered from the town of Mazar-e-Sharif and its surroundings in Northern Afghanistan. From these, 17972 cases (84 %) were detected as *Leishmania major* (rural form), 3433 cases (16%) as *Leishmania tropica* (urban form) and 15 cases of Lupoid form. In our results we recorded the high incidence of positive cases (mainly rural form) during the autumn that can be explained by the incubation period of leishmaniasis and the activity of vectors especially in summer months.

# 31

Molecular Polymorphism of *Tetratrichomonas gallinarum*. I. CEPICKA, K. KUTISOVA and J. FLEGR, Department of Parasitology, Charles University, 128 44 Prague, Czech Republic.

Tetratrichomonas gallinarum is a widespread parasite of birds, mainly Galliformes and Anseriformes. This species usually infects caecum, but it was found also in beak, salpinx and different visceral organs. Several strains were also isolated from human mouth and lungs. There were originally described several Tetratrichomonas species from different avian hosts, but on the basis of morphological and infection studies only one species is presently considered to be valid. We performed the phylogenetic analysis with 19 T. gallinarum strains, including two human strains and 17 strains isolated from 8 different avian species. The sequences of 5.8S rRNA with the flanking areas ITS1 and ITS2 (ITS) of these 19 strains were determined and used for construction of the phylogenetic tree by the Neighborjoining and Maximum parsimony method. The strains formed 5 clusters A-E; sequences in each cluster were identical. Human isolates were placed in clusters A and B. The sequence homology among clusters A, B and C (92.9% - 95.4%) was higher than the sequence homology among four valid Trichomonas species (86% - 89%). However, the sequence homology of clusters D or E to clusters A, B and C, as well as the sequence homology between clusters D and E was unexpectedly low (78.2% - 91%). We also sequenced and analyzed the gene for 16S rRNA from one strain of each cluster. Again, the sequence homology between clusters D and E, as well as between cluster D or E and clusters A, B and C, was lower than the sequence homology between two Trichomonas species. Therefore we suggest that our strains of Tetratrichomonas gallinarum could represent at least three cryptic species.

# 32

Malic Enzymes of *Trichomonas vaginalis*: Purification, Function and Phylogeny. P. DOLEZAL\*, J. TACHEZY\*, P. PROOST\*\* and I. HRDY\*, \*Department of Parasitology, Faculty of Science, Charles University, 128 44 Prague 2, Czech Republic, \*\*Rega Institute for Medical Research, Department of Microbiology and Immunology, Leuven, Belgium.

*Trichomonas vaginalis* possesses two types of malic enzymes: hydrogenosomal NAD(P)-specific and cytosolic NADPspecific malic enzyme. Hydrogenosomal type is a homotetramer of the 60 kDa subunits. It utilizes NAD<sup>+</sup> preferentially to

Improved Methods for Preparation of a 25 kDa Ciliary Glycoprotein of *Tetrahymena thermophila*. A. W. WAY and L. A. HUFNAGEL, University of Rhode Island, Kingston, RI.

Concanavalin A (Con A)-binding membrane proteins may have an important role in initial recognition and formation of the conjugation junction between mating-competent T. thermophila cells. To further investigate this, methods were developed for increased yield of a ciliary membrane Con A-binding protein, in preparation for its further analysis. Variables examined included methods to achieve high yields of isolated cilia, affinity column chromatography techniques, alternative protease inhibitors, and gel spin columns to remove salts and low molecular weight polypeptides. When Con A affinity chromatography was optimized, a polypeptide of approximately 25 kDa was obtained from detergent (Nonidet P-40)-solubilized ciliary membranes of nonstarved (mating inactive, non-initiated) cells. Consistent with previous results, this polypeptide was either down regulated or no longer able to bind Con A in starved (mating competent, initiated) cells. Blotting verified that the polypeptide is Con A-binding. We estimate that, using these methods, from one liter of late exponential phase cells grown on enriched proteose peptone (~ 16-20 ml packed cells), approximately 0.1 mg of the 25 kDa glycoprotein can be obtained. These experiments establish procedures for providing sufficient quantities of the 25 kDa protein for further studies, including antibody production, isoelectric focusing, and sequence analysis.

# 29

Effects of Latrunculin B on *Tetrahymena* Mating. R. V. ZACKROFF\* and L. A. HUFNAGEL\*\*, \*Massachusetts College of Pharmacy and Health Sciences, Boston, MA, \*\*University of Rhode Island, Kingston, RI.

Early events in Tetrahymena mating include tip transformation, in which the anterior ends of the cells become altered in preparation for mating, and loose pairing, which may involve both heterotypic and homotypic interactions at cell surface sites not restricted to the transformed tips. Tip transformation and loose pairing appear to precede tight heterotypic pairing at the transformed tips. To shed further light on the mechanism of these early events, we investigated the effects of latrunculin B, an anti-actin drug that inhibits Tetrahymena phagocytosis (a well characterized, actin-dependent process) at sub-micromolar concentrations. The rate of stable pair formation was significantly inhibited by sub-micromolar (4-8 x 10<sup>-7</sup> M) concentrations of latrunculin B. Micromolar concentrations of latrunculin B strongly inhibited the rate of pairing as well as tip transformation. However, the first observable pairs appeared nearly simultaneously in drug-treated samples and controls. These results suggest that tip transformation is dependent on actin dynamics, and that actin may be involved in other aspects of stable pair formation as well.

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NADP<sup>+</sup> and supplies hydrogenosome with pyruvate for further catabolic processes. In this study we focused on NADP-specific malic enzyme from the cytosol. One of the several present isoforms was purified to homogeneity by liquid chromatography. Molecular weight determination of the native purified isoform suggested an unusual dimeric arrangement of the 42 kDa subunits. The enzyme utilizes exclusively NADP+ (Km, 2,8 µM) with pH optimum between 7,5-8,5. The main function of the cytosolic malic enzyme is probably production of NADPH which is then utilized in the formation of the major glycolytic end product, glycerol. Moreover, together with the consecutive action of phosphoenolpyruvate carboxykinase and malate dehydrogenase the cytosolic malic enzyme forms a pathway that bypasses pyruvate kinase reaction and transfers reducing equivalents from NADH to NADP+. We cloned and sequenced the complete gene of NADP-specific malic enzyme. The coding region is preceded by initiator element while 3'-flanking region contains polyadenylation signal, thus corresponding to known arrangement of transcription unit in T. vaginalis. Amino acid sequence comparisons with homologous proteins revealed eubacterial features of cytosolic malic enzyme and indicated totally different evolutionary origin of both types of T. vaginalis malic enzymes. Hydrogenosomal type appears to be one of the most divergent eukaryotic malic enzyme. Apart from their obvious role in metabolism of T. vaginalis, several studies have described specific extracellular release of the malic enzymes and suggested their adhesive function. To verify this hypothesis, we analyzed the proteins excreted by T. vaginalis into Doran medium. In addition to described secretion of both malic enzymes we detected other cytosolic (LDH, MDH) and hydrogenosomal (STK) proteins as well. We also examined effect of T. vaginalis secreted proteases on the activity of MDH, LDH and malic enzymes. In cell-free supernatants obtained after onehour cell incubation both malic enzyme remained active over four hours while activities of MDH and LDH were undetectable after 30 minutes. We propose that extracellular localization of malic enzymes does not indicate their adhesive function but rather reflects unspecific exocytosis of cell content in combination with resistance of malic enzymes to proteolytic degradation.

# 33

Phylogenetic Position and Relationship of Flagellates of the Genus *Monocercomonas* from Different Host Species. V. HAMPL, I. CEPICKA, J. KULDA, J. TACHEZY. and J. FLEGR, Department of Parasitology, Faculty of Science, Charles University, 128 44 Prague, Czech Republic.

The genus Monocercomonas Grassi, 1879 (order Trichomonadida) comprises more than 20 species mostly parasitic in reptiles but also in other vertebrates and in insects. Important morphological feature of this genus is the absence of costa and undulating membrane, characteristic structures of a typical trichomonad cell. Family Monocercomonadidae Kirby, 1944, established for trichomonads with similarly reduced cell morphology, comprises besides parasitic also free-living genera (Pseudotrichomonas, Ditrichomonas, Monotrichomonas). This family was regarded to be the most primitive group of trichomonads from the evolutionary point of view. However, results of sequencing of 16S rRNA of some species suggested that they probably represent higher branches in the trichomonad tree. Moreover, they indicate that the free-living genera are unrelated to the genus Monocercomonas. Here we sequenced the gene for 16S rRNA of two isolates of Monocercomonas ruminantium from cattle and eight isolates of Monocercomonas sp. from different reptile species. Strains from reptiles formed one clade with the bootstrap value 100 in the phylogenetic tree of trichomonads. On the other hand sequences of the two isolates of *Monocercomonas ruminantium* were placed with 100% bootstrap support among the free-living genera of trichomonads, i.e. into a branch of species unrelated to other representatives of the genus *Monocercomonas*. The sequences of these isolates differed only in one nucleotide and were closely related (97.1 %) to the sequence of *Pseudotrichomonas keilini*. Our results strongly suggest that taxonomic classification of *Monocercomonas ruminantium* should be revised. Furthermore, close relationship of this parasitic species with free-living trichomonads pose interesting questions regarding the evolution of parasitism in this group.

#### 34

What is the Cause of the Deteriorated Performance in Subject with Latent Toxoplasmosis? J. HAVLIÇEK\*, J. KLOSE\*\*, M. PREISS\*\*, P. KODYM\*\*\* and J. FLEGR\*, \*Department of Parasitology, Faculty of Sciences, Charles University, Prague, Czech Republic, \*\*Department of Psychology, Central Military Hospital, Prague, Czech Republic, \*\*\*National Diagnostic Laboratorz for Toxoplasmosis, National Institute of Health, Prague, Czech Republic.

The ability of parasitic protozoan Toxoplasma gondii to change behaviour of its host is well documented. In mice, the infection causes an impaired motor performance, deficit in learning capacity and lower ability to discriminate between familiar and novel surroundings, and longer reaction times. Infected rats have higher activity levels, lower neophobia and reduced learning capacity. In human hosts, shifts in psychological profiles of the infected subjects were observed. On the other hand, there is only one study concernig the effect of infection on performance. The simple reaction time test showed that infected subjects had prolonged reaction times in comparison with Toxoplasma-free subjects. The aim of the present study is to reveal which psychomotor functions are particularly influenced by the infection. The total sample consisted of 551 conscripts (26.3 % Toxoplasma positive). Psychomotor testing was performed in Central Military Hospital, Prague, during a routine psychological session. Performance of infected and noninfected subjects in 3 series of tests was compared by ANOVA with repeating measures. Statistical analysis revealed that Toxoplasma negative subjects performed significantly better (F(1; 549) = 10.64; p = 0.001) than the *Toxoplasma* positive ones. Because of the existence of negative correlation of IQ with the infection, this variable was added as covariate into the analysis. However, the differences in performance were still highly significant (F(1; 549) = 0 8.04; p = 00.005). Post hoc analyses of tests dynamics suggest that the deterioration in performance in the infected subjects is due to loss of concentration or fatigue rather than memory or learning capabilities.

### 35

Coccidian Parasites of the Genus *Eimeria* (Apicomplexa: Eimeriorina) in Ranid Frogs in the Czech Republic. M. JIRKU\*, M. VESELY\*\* and D. MODRY\*\*\*\*\*, \*Department of Zoology, Faculty of Natural Sciences, Palacky University, 771 46 Olomouc, Czech Republic, \*\*Department of Parasitology, University of Veterinary and Pharmaceutical Sciences, Palackeho 1–3, 612 42 Brno, Czech Republic, \*\*\*Institute of Parasitology, Academy of Sciences of the Czech Republic, Branisovská 31, 370 05 Ceske Budejovice, Czech Republic.

Until now about 45 coccidia species (Apicomplexa: Eimeriidae, Sarcocystidae) parasiting anuran amphibians are known. The aim of presented research was to study the prevalence of coccidian species in ranid frogs in some localities in the Czech Republic. Hyaloklossia lieberkuehni Labbe, 1894 and Eimeria sp. were found in R. kl. esculenta, whereas the examination of R. temporaria and R. arvalis revealed E. ranae Dobell, 1908 to be the only coccidian species parasitising these two ranid hosts. E. ranae was relatively common parasite of R. temporaria, reaching a prevalence of 57.5% in adults. Examination of tadpoles of R. temporaria revealed the presence of E. ranae in their faeces. Consequent histological examination confirmed stages of merogony and gamogony in the intestine of 13% of tadpoles in population examined. Presence of oocysts in both tadpoles and adults of R. temporaria indicate the completion of the life cycle even before the metamorphosis. Additionally, the infectivity of adult frog-originated oocvsts for tadpoles was confirmed by results of cross-transmission experiments. However, there was no evidence of any coccidian parasites to be present in freshly metamorphosed specimens of R. temporaria in nature. Morphologically identical oocysts were found also in adults of R. arvalis and possible conspecificity of coccidia from R. arvalis and R. temporaria, as well as the natural life cycle of E. ranae is discussed.

# 36

Use of Random Amplified Polymorphic DNA (RAPD) Analysis for the Identification of *Giardia intestinalis* Subtypes. J. SEDINOVA\*, J. FLEGR\*, P. L. EY\*\* and J. KULDA\*, \*Department of Parasitology, Faculty of Science, Charles University in Prague, Vinicna 7, 128 44 Prague 2, Czech Republic, \*\*Department of Molecular Biosciences, Adelaide University, Adelaide SA 5005, Australia.

The random amplified polymorphic DNA (RAPD) method was used to investigate genetic polymorphisms among 25 isolates of Giardia intestinalis and to assess the utility of RAPD for subtype detection and genealogical analysis. Using data obtained for 6 human and 19 animal-derived isolates in polymerase chain reactions with 13 different primers, phylogenetic trees were constructed and bootstrap values computed by the program FreeTree. Three major clades were distinguished, corresponding to previously defined genetic assemblages A, B and E. The purported specificity of assemblage E genotypes for artiodactyl hosts was supported. Assemblages A and B showed wide host spectra, including human and animal hosts. The identity of RAPD patterns obtained from 14 clones derived from one G. intestinalis isolate (P15) indicated that the isolate was genetically homogenous. No correlation was found between the genotype of analysed isolates and the presence or absence of the double-stranded RNA Giardiavirus. The results indicate that RAPD banding data provide reliable genetic information that can be used for both 'fingerprinting' and genealogical purposes.

### 37

*Leishmania tropica*: Field Study in the Endemic Focus in Turkey and Laboratory Transmission from Asymptomatic Hosts and Non-lesion Sites. M. SVOBODOVA and P. VOLF, Department of Parasitology, Charles University, Vinicna 7, 128 43 Prague 2, Czech Republic.

Sandflies were studied in Urfa, Turkey—a focus of *L.tropica*. Light and rodent traps were placed in the endemic quarters. *Phlebotomus sergenti* caught was twice as many in number as *P.papatasi*. These 2 species constitute 99% of sandflies. The

male:female ratio was 1.4 for P.sergenti and 0.9 for P.papatasi. Both species were most abundant in stables followed by cellars, and less so in rooms, yards and on roofs. P.papatasi was relatively more numerous in stables, while P.sergenti in rooms. Sources of bloodmeals were determined by ELISA using anti-IgG. The bloodmeals originate from several vertebrates including rodents. House mice (Mus domesticus) and black rats (Rattus rattus) were abundant and caught in sandfly-infested houses. Black rats were susceptible to infection with L.tropica. The parasites produced no skin lesion, but persisted at the inoculation sites of ear and footpad. P.sergenti fed on the rat ears acquired infection. BALB/c mice developed lesions after inoculation with L.tropica, and the infection eventually visceralized. Feeding on non-lesion sites resulted in 20% infected P.sergenti. Thus, P.sergenti can acquire infection on inoculation site of an asymptomatic host and on non-lesion site of a symptomatic host, which is important in the epidemiology of the disease.

### 38

A New Paraxonemal Protein in *Giardia intestinalis*. P. TES-AROVA and E. NOHYNKOVA, Department of Tropical Medicine, 1st Faculty of Medicine, Charles University, Studnickova 7, 128 00 Prague, Czech Republic.

Paraxonemal structures of different morphological pattern parallel flagellar axonemes in cells of several unrelated groups of protists, including retortamonads, diplomonads, parabasalids, kinetoplastids etc. Of these, a paraflagellar rod (PFR) of trypanosomatids is well characterized while little is known about others. The PFR consists of two major closely related proteins, PFR1 and PFR 2, which together with some minor proteins compose a highly ordered paracrystalline structure alongside the flagellar axoneme. We used two monoclonal antibodies, L13D6 and L8C4, specific for the major paraflagellar proteins of Trypanosoma brucei (PFR-A and PFR-C) to test whether related proteins are present in paraxonemal structures found in Giardia intestinalis, G. muris and Tritrichomonas foetus. In Giardia, different structures accompany ventral flagellar axonemes and intracytoplasmic portions of axonemes of anterolateral and posterolateral flagella, in T.foetus, an undulating membrane associated recurrent flagellum contains a rod-like paracrystalline structure. In indirect immunofluorescence, the antibody L13D6 which recognizes both T.brucei PFR-A and PFR-C decorated specifically intracytoplasmic portions of both axonemes of anterolateral flagellar pair in G.intestinalis. Doublelabel staining with monoclonal antibody against alpha tubulin showed that the L13D6 antibody label was localized anteriorly to the axonemes in close proximity to axonemal microtubules. During cell division, the paraxonemal label disappeared in prophase in accord with flagellar rearrangement and reappeared in telophase when daughter anterolateral flagella assumed the typical interphase arrangement. Surprisingly, no label was detected in any phase of G.muris or T.foetus cell cycles. The antibody L8C4, specific for the PFR-A only, did not recognize any epitope in any of the flagellates tested.

Cyclophilins of *Trichomonas vaginalis*. V. VARGA\*, M. DUCHENE\*\*, J. TACHEZY\*, I. HRDY\*, J. KULDA\* and T. THALHAMMER\*\*, \*Department of Parasitology, Faculty of Science, Charles University in Prague, Vinicna 7, 128 44 Prague 2, Czech Republic, \*\*Department of Pathophysiology, University of Vienna, Waehringerguertel 18–20, A-1090 Vienna, Austria.

Cyclosporin A (CsA), initially developed as an immunosuppressive drug, possesses activity against various protozoan parasites in a species and/or stage specific manner. CsA binds to the high affinity intracellular receptors, cyclophilins (Cyps). The cyclophilins display the peptidyl-prolyl cis/trans isomerase activity and accelerate the rate-limiting step in the folding of target proteins. If CsA binds to Cyp, the CsA/Cyp complex inhibits calcineurin-mediated dephosphorylation of transcription factors, which in their phosphorylated form are unable to translocate in the nucleus. The present study was undertaken to characterize Cyps in Trichomonas vaginalis and to test the susceptibility of the parasite to CsA and related compounds. Using a CsA-affinity column an 18kDa protein was isolated from a cytosolic fraction of T. vaginalis. The protein was unglycosylated and peptide sequencing showed its high homology to Cyps of other parasites. The protein possessed peptidyl-prolyl cis/ trans isomerase activity, inhibitable by CsA (IC50  $\sim$  10nM), indicating that this protein belongs to cyclophilins. Furthermore, the isoelectric focusing revealed two isoforms with an isoelectric point around 8.6, as observed for other cytosolic Cyps. Exposure of T. vaginalis to CsA for 24 and 48 hrs showed that CsA is toxic to the parasite at the minimal lethal concentration (MLC) of 60 µM. Interestingly, another CsA-derivative, the SDZ PSC833, which does not bind to the human cytosolic Cyp, was more effective than CsA (MLC about 7 μM). It indicates, that Cyp binding and inhibition of peptidylprolyl cis/trans isomerase activity might not be the only mechanism of the CsA action against T. vaginalis. Further studies are required to elucidate the physiological function of this novel T. vaginalis cyclophilin and to evaluate its potencial as a target for therapy.

#### 40

Development of Avian Trypanosomes in Mosquitoes. J. VO-TYPKA\*.\*\*, M. SVOBODOVA\* and P. VOLF\*, \*Department of Parasitology, Faculty of Science, Charles University, 128 44 Prague, Czech Republic, \*\*Institute of Parasitology, Czech Academy of Sciences, 370 05 Ceské Budjovice; Czech Republic.

Parasitic flagellates of the genera Trypanosoma and Leishmania have a two-host life cycle and alternate between vertebrate and insect hosts. Transmission of the parasite into the insect vector is due to uptake of flagellates with peripheral blood. However, transmission to the vertebrate host may occur in several ways, for example, by inoculation with insect saliva or contamination with insect feces. The location of parasites in the insect digestive tract or in the salivary glands is critical to the mechanism of transmission to the vertebrate host. Avian trypanosomes (strain CUL1, which originates from naturally infected Culex pipiens pipiens) were used to infect laboratory reared female mosquitoes (Culex pipiens quinquefasciatus). Infected mosquitoes (16 days post infection) were dissected and heavy infections of trypanosomes were found in the division between the foregut and the thoracic midgut. The other parts of the digestive tract including salivary glands were parasitefree. Semithin and thin cross-sections of the fore part of the alimentary tract containing trypanosomes were analyzed by light and electron microscopy, respectively. The parasites observed were in close contact with chitin in the cuticular lining of the stomodeal valve only; the region of epithelium covered by microvilli was not colonized. The adhesion of the parasite to this cuticle-lined region occurs by the formation of zonal hemidesmosome-like plaques at the extremities of the expanded flagella of epimastigote. The high number of trypanosomes in the anterior part of the thoracic midgut creates a plug, which could result in the regurgitation of flagellates into the vertebrate host with a backflow of ingested blood during sucking. Additionally, some damage to the chitin layer or impairment of the stomodeal valve motility could help such a transmission process. The development of avian trypanosomes in mosquitoes is similar to that of the leishmanias in sand flies and therefore could represent a new model for the investigation of host-parasite relationships.

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# 41

Environmental Effects on Cholinergic Molecule Activity in Protists. A. AMAROLI\*, C. FALUGI\*\*, L. SANNA\*, V. EVANGELISTI\*\*\*, A. VIARENGO\*\*\* and M.U. DELMON-TE CORRADO\*, \*Dipartimento per lo Studio del Territorio e delle sue Risorse, \*\*Dipartimento di Biologia Sperimentale, Ambientale, Applicata, Università di Genova, 16132 Genova, \*\*\*Dipartimento di Scienze e Tecnologie Avanzate, Università del Piemonte Orientale, 15100 Alessandria, Italy.

It is well known that neurotoxic drugs, such as organophosphate (OP) and carbamate (CB) compounds, are currently employed in agricultural sites, because of their insecticide activity. The first target of these compounds is the cholinergic neurotransmitter system, by inhibiting cholinesterase activity. Therefore, this enzyme activity is usually exploited as a biomarker of neurotoxic drugs. Recently, we found acetylcholinesterase (AChE) activity in Paramecium primaurelia and its role was inferred in modulating the pre-conjugant cell-to-cell interactions. The results of our investigations extended to Dictyostelium discoideum by histochemical, electrophoretic, and spectrophotometric methods showed the presence of a cholinesterase (ChE) activity able to cleave both the acetyl-beta-methyltiocholine iodide and propionyltiocholine iodide substrates, sensitive to neurotoxic drugs, and possibly involved in regulating cellto-cell interactions during aggregation. In this study, we characterized the D. discoideum ChE activity by evaluating spectroscopically the effects of specific ChE inhibitors, such as iso-OMPA, BW, and eserine, as well as by a spectrophotometric analysis performed under varying conditions of chemico-physical parameters, i.e. pH and temperature. Furthermore, we checked the chance to exploit P. primaurelia as a bioassay for the pre-chemical screening of fresh water environments in relation to the occurrence of neurotoxic drugs. The presence of propionylcholinesterase (PrChE) activity was histochemically revealed and the spectrophotometric analysis showed significantly higher AChE activity than PrChE activity. Exposure both of AChE and PrChE to 10-6 M Diazinon or 10-6 M Carbaryl, the active principles of OPs or CBs, respectively, resulted in significant inhibition of AChE activity.

# 42

Evaluation of a Rapid Immunochromatographic Test for the Serodiagnosis of Visceral Leishmaniasis. O. BRANDONISIO, L. FUMAROLA, D. LEOGRANDE and P. MAGGI\*, Dipartimento di Clinica Medica, Immunologia e Malattie Infettive, Se-