*Isospora belli* is a cause of gastroenteritis in humans. An intriguing feature of isosporiasis in HIV/AIDS patients is that "monozoic cysts" (Dubey & Frenkel, 1972, J. Infect. Dis. 125: 69–72) can sometimes be found extra-intestinally in persons who are shedding *I. belli* oocysts in their faeces. These singly-occurring, extra-intestinal organisms may be responsible for re-activation of the gut infection. The unique ultrastructure of the "monozoic cyst" was first described by Mehlhorn & Markus (1976, Z. Parasitenk. 51: 15–24). A prominent feature is the crystalloid body, which is characteristic of some coccidian sporozoites and post-divisional stages. Considering that extra-intestinal forms of *I. belli* and other species of *Isospora* of mammals have not yet been seen to divide, the question arises as to whether "monozoic cysts" are dormant sporozoites that have emerged from ingested oocysts (Markus, 1991, Med. Hypoth. 35: 278). However, the fact that such large numbers of these parasites can occur in various extra-intestinal tissues in cases of HIV/AIDS, suggests that swallowing of oocysts may not be their origin. It is assumed that the "monozoic cysts" in HIV/AIDS are those of *I. belli* rather than of isosporan species of other mammals, but this is not certain (Velásquez et al., 2001, Hum. Pathol. 32: 500–505). The function of "monozoic cysts" of *I. belli* in HIV/AIDS remains unknown, as does their origin and nature in respect of the coccidian life cycle.

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## Czech Section Society of Protozoologists
## 33rd Annual Meeting
## May 12–16, 2003
## Sec, Czech Republic
## (Abstracts 67–74)

## 67

The development of a precocious strain of *Eimeria necatrix*. M. HORTVÍKOVÁ, BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs, Jilove u Prahy, Czech Republic.

A precocious line of the coccidium *Eimeria necatrix* (MH/2002) with an abbreviated life cycle was derived from a parent strain (UK/NH/94/95) obtained by crossing Czech and English strains of this species. The life cycle was abbreviated by repeated inoculation of chickens with the first oocysts produced in previous infection of chickens. The earliest oocysts recovered in the first passage were used for infection of birds for the second passage, and so on. Oocysts were harvested from caecal content. The prepatent period (time from infection to excretion of oocysts) of the parasite was reduced by 26 hours (from 148 to 122) after 12 passages. The patogenicity of the attenuated line was considerably reduced in comparison with the parent strain. The dose of 105 oocysts/bird of the parent strain caused 80% mortality due to coccidiosis, whereas the same dose of oocysts of the precocious line caused 0% mortality. The immunogenicity of the precocious line *E. necatrix* against parent strain was maintained. Even a dose of 50 oocysts/bird of the precocious line started immunity that protected the chickens against challenge of 104 oocysts of the parent strain.

## 68

Development of PCR for the detection of chickens *Eimeria* species. Z. HOUDEK, R. ORAVEC, P. TREFIL, M. HORTVÍKOVÁ and T. MIKU, BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs, a. s. 254 49 Jilové u Prahy, Czech Republic.

The PCR was performed using DNA isolated from $10^7$ oocysts cracked with glass beads (Ø 0.5 mm) (according to Shirley et al. 1995 and Schnitzler et al. 1999). The oocysts were purified from droppings (*Eimeria maxima, E. praecox*) or from the caecal content (*E. tenella, E. necatrix*) of chickens inoculated with oocysts of the given coccidian species. Species-specific primers pairs for amplification of the internal transcribed spacer 1 (ITS1) regions of each *Eimeria* species were used. Specific fragments were identified by agarose electrophoresis analysis. In order to verify sensitivity of PCR, different numbers of intacted coccidian oocysts were used (1000, 100, 10 or 1 respectively). Tween 20 in the final concentration 0.8, 0.4 or 0.0% respectively was added to the oocysts suspension to enable their cracking. In order to crack the oocysts walls, the suspension was ten or twenty times warmed up to 96 °C for 5 min and cooled down to 4 °C for 5 min. Alternatively, the same numbers of sporozoites released from the *E. tenella* oocysts in vitro were tested in the PCR using the same concentration of Tween 20 (0.8, 0.4, or 0.0% respectively) and warming-cooling cycles as we described above. The specific fragments were recovered only in reactions with 1000 and 100 sporozoites. Tween 20 in concentration of 0.4 or 0.8% had no effect on the reaction. This method is able to detect 100 sporozoites released from the oocysts.

## 69

Distribution of ciliates of different feeding specialisations in the water bodies of different trophy. M. MACEK*,**, K. SIMEK** and A. L. VAZQUEZ*, *Universidad Nacional Autonoma de Mexico campus Iztacala, Av. de los Barrios 1, los Reyes Iztacala, Tlalnepantla, Mexico, ** Hydrobiological Institute, Academy of Sciences, Ceske Budejovice, Czech Republic.

Extended dataset of the structure of ciliate assemblages covering different trophic status of water bodies was analyzed. Ciliate taxa were identified using semi-quantitative protargol staining approach; feeding patterns were detected using fluorescence microscopy methods. Mean water column abundance and biomass (depth-weighted averages) was used to calculate data-weighted seasonal averages. Cluster analysis was performed using the method of Euclidean distances without a data transformation. Abundance- or biomass-percentage distribution of ciliates with distinct feeding patterns and total abundance or biomass of ciliates, respectively, was employed. The whole-season studied oligotrophic lakes were represented by the above-timberline lakes located at different latitudes (in Norway, Scotland, Slovakia and Russia), mountain-forest acidified lakes (in the Czech Republic), and high altitude athalassohaline lakes (in Mexico). The analysis was performed also with seven annual datasets of two meso- to eutrophic reservoirs (in the Czech Republic) and with forty short term-studied or one point-sampled from several European lakes. We did not find any remarkable differences in the ciliate distribution studied between the above timberline- and forest-surrounded lakes. The oligotrophic lakes were clustered into four basic groups: Oligotrichous mixotrophs (genera *Pelagostrombidium, Limnostrombidium, Rimostrombidium*) were the most prominent in remote pristine lakes (1) where a balanced composition with gymnostomatids (genera *Askenasia, Mesodinium*) was observed meanwhile prostomatids (particularly *Urotricha* spp.) numerically prevailed in others, dominating absolutely in acidified lakes; (2) the significant contribution of ciliate picoplankton-feeders (minute oli-

gotrichs of genera *Halteria* and *Rimostrombidium*; scuticociliates; peritrichs of genera *Rhabdostyla, Cothurnia*) was recorded but they prevail within the water column scarcely, e.g. in Mexican lakes; (3) gymnostomatids (*Mesodinium* sp.) prevailed absolutely only in several lakes; (4) anoxic bottom affected the clustering (hymenostomatids; genera *Loxodes, Caenomorpha*) due to their high individual biomass. Reservoir assemblages partly clustered with the group (1), however, presented unsteady annual distribution pattern.

## 70

Methods of analysis and 3D imaging of protozoan cytoskeletal structures obtained by confocal microscopy. J. NOVY*, J. TOMAS* and R. JANISCH**, *Institute of Mathematics, Faculty of Mechanical Engineering, University of Technology, **Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic.

The cytoskeleton image provided by fluorescence microscopy methods in large protozoan cells does not correspond to the real spatial arrangement of this structure. The fluorescence imaging of microtubular cytoskeletons in the infusorian ciliate *Paramecium caudatum* cannot reveal the very complex system of microtubules constituting the surface cortex including an intricate oral apparatus. Similarly, the role of microtubules in the endonuclear mitosis of micronuclei and in segregation of the macronuclear genomes or their role in the mechanical support of contractile vacuoles and governing the lysosomal vesicle pathway from the cytostome via food vacuoles to the cytoproct can hardly be elucidated using fluorescent imaging. However, confocal microscopy brought about great progress providing images that can be further enhanced with the use of numerical methods that adapt the final image to our spatial perception of the macroscopic world. The principles of adaptive numerical processing and the results obtained in selected microtubular systems of *Paramecium* were as follows: The input data acquired with a Fluoview 2 Olympus confocal microscope were processed using two different approaches. The first led to 2D images with enhanced contrast with respect to partial translucency of the objects, the second provided 3D reconstructions of the cytoskeleton. The resulting 2D images were composed using a summation method. The contrast enhancement was achieved by a modification of the Fourier spectrum using a high-pass filter with suppressed adaptive noise. The noise filtering is based on statistical properties calculated from the processed image background. The histogram equalization was used for further enhancement of images, with a resulting uniform distribution of intensity. Techniques of adaptive kernel convolution were employed for 3D reconstruction. For a more simple analysis of the cytoskeletal structures, two different models of 3D reconstruction were developed. Both of them employ linear perspective projection. The first method was based on a particle model and was used for an overall analysis of the cytoskeleton. The second one included the real-time calculated projection with the use of Goraud shading and a suitable illumination model to analyze selected parts of the cytoskeleton.

## 71

Selection and characterization of a precocious line of rabbit coccidium *Eimeria flavescen.* M. PAKANDL*, F. CERNÍK** and Z. HOUDEK**, *Institute of Parasitology, Academy of Sciences, Ceské Budejovice, Czech Republic, **BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs, Jilove u Prahy, Czech Republic.

Although precocious lines (PL) of several species of rabbit coccidia has been already obtained, until now, no PL has been derived from the highly pathogenic species *Eimeria flavescens.* In our experiment, a precocious line of *E. flavescens* was derived from a parent strain by selection for early development of oocysts in rabbits and after 12 consecutive passages in animals. The time from inoculation to appearance of the first oocysts in cecal content was shortened by 60 hours. The oocysts of the original strain (OS) and PL did not differ in their morphology. The reproductive potential and pathogenicity were assessed in an experiment involving seven groups of six animals each: uninfected control and inoculated with 500, 5.000 and 50.000 oocysts of parent strain or PL respectively. Unlike OS, the infective doses of 500 and 5.000 oocysts of PL were not sufficient to obtain maximal oocyst yield. Thus, the reproductive potential of PL was markedly reduced. The weight gain was affected in all the groups infected with OS and the group infected with 50.000 oocysts exhibited severe diarrhea and weight loss from 8 to 16 days past inoculation (DPI). In contrast, only the group infected with 50.000 oocyst of PL showed moderate diarrhea and reduction of weight gain for about three days. As the pathogenicity of PL is sufficiently reduced, it is promising for coccidiosis control. Immunogenicity of PL and a possible method of vaccination are subject of a future study.

## 72

Specificity of sand fly salivary antigens and host antibody response. I. ROHOUSOVA and P. VOLF, Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic.

Sand fly salivary components are known to interfere with the immune response of the host and enhance the parasite infection. However, mice immunised with the sand fly saliva develop an antibody response, which inhibits the enhancing effect. The aim of this study was to compare salivary antigens in various sand flies and characterize the antibody response of individuals from endemic area. Species-shared and species-specific antigens in salivary gland lysate (SGL) of *Phlebotomus papatasi* (origin Turkey), *P. sergenti* (Turkey) and *Lutzomyia longipalpis* (Brazil) were characterized by dotblots and immunoblots using sera of animals bitten by one of these sand fly species. All sera strongly reacted with homologous antigen. Partial cross-reactivity was found only between *P. papatasi* and *P. sergenti.* High specificity of antibody response in experimental animals encouraged us to characterize antibodies in sera from samples obtained in endemic area. ELISA tests were performed with human sera collected in endemic area of *Leishmania tropica* in Sanli Urfa (Turkey), where *P. sergenti* and *P. papatasi* are two dominant species (99% of local sand fly population). High levels of specific anti-*P. papatasi* and anti-*P. sergenti* IgG were detected in sera from Sanli Urfa. No positive reaction was observed when *L. longipalpis* saliva was used as an antigen. Immunoblot techniques revealed similar results: sera from Sanli Urfa recognized up to 10 protein bands in the homologous antigen (SGL of *P. papatasi* and *P. sergenti*) while all control sera showed only very weak reaction or were negative. In addition, antibodies from a different source (human and mouse sera) recognized similar *P. papatasi* antigens but with different intensity. Human sera reacted mostly with 30 kDa antigen that is similar to *Aedes aegypti* D7 protein, while antibodies from mouse sera recognized preferentially the 42 kDa Yellow protein. In summary, most components of sand fly saliva are highly immnunogenic, but species-specific. These data are important for characterization of salivary proteins for feasible transmis-

sion blocking vaccine and for understanding of the interplay between blood feeding insects and their hosts.

## 73

Prevalence of *Neospora caninum* antibodies in zoo animals in the Czech Republic. K. SEDLAK, Department of Virology and Serology, State Veterinary Institute Prague, Prague, Czech Republic.

*Neospora caninum* is an apicomplexan parasite that causes neuromuscular disease in dogs and abortions in cattle. Little is known about the prevalence of antibodies to this parasite in zoo animals. Sera from 242 animals from 9 Czech zoos were tested for antibodies to *N. caninum* and *Toxoplasma gondii* by indirect fluorescent antibody test. Antibodies to *N. caninum* were found in 8 zoo animals (3.3%) with titres 1:40 (1 african buffalo), 1:80 (1 wolf, 1 cheetah, 1 eland), 1:160 (1 cheetah, 1 sitatunga), 1:320 (1 wolf), and 1:640 (1 maned wolf). Antibodies to *T. gondii* were detected in 80 zoo animals (33%), with titers ranging from 1:40 to 1:10240. The results of this study indicate that zoo animals in the Czech Republic have a history of increased exposure to *T. gondii* than to *N. caninum* in this type of environment.

## 74

*Phlebotomus arabicus*—a new vector of *Leishmania tropica.* M. SVOBODOVA*, J. VOTYPKA*, J. PECKOVA*, R. L. JACOBSON** and A. WARBURG**, *Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic, **Department of Parasitology, Hebrew University, Jerusalem, Israel.

Sand flies (Diptera: Psychodidae) were collected in four villages endemic for *Leishmania tropica* in the Galilee region of Northern Israel during eight nights in September 2002 (total 154 traps/nights). CDC light traps were placed overnight between piles of boulders surrounding the villages, in orchards, and near houses in the periphery of the villages. Of the 413 females captured, 40% were *Phlebotomus (Larroussius) tobbi,* 32% *P. (Paraphlebotomus) sergenti,* and 15% *P. (Adlerius) arabicus. P. (Adlerius) simici, P. (Larroussius) perfiliewi* and *P. (Phlebotomus)* papatasi together represented ~10% of the catch. In males (N = 1077), the relative numbers were similar with the addition of 1% *P. (Laroussius) syriacus.* Sand fly females were dissected and examined microscopically for presence of promastigotes. Four females of *P. arabicus* (7%) and one of *P. sergenti* (1%) harbored promastigotes in their gut. The infections were heavy and mature in *P. arabicus,* while only moderate in *P. sergenti.* Parasites were characterized by isozyme electrophoresis, two immunological and three PCR-based methods. Isolates were antigenically similar to *L. major* but molecular and biochemical tests showed that they belonging to a newly reported zymodeme of *L. tropica* separable from all known *L. tropica* isolates using two different PCR–based methods. Laboratory-bred *P. arabicus* and *P. sergenti* originating from the endemic area were fed on skin lesions of golden hamsters infected with one of the isolates. More then 17% of the *P. arabicus* females became infected (N = 6), but none of the *P. sergenti* (N = 157). This is the first report of *P. arabicus* naturally infected with *Leishmania,* strongly suggesting that this species is the vector of *L. tropica* in the Galilee region of Northern Israel.

# German Section Society of Protozoologists 22nd Annual Meeting March 2003 Nijmegen, The Netherlands in connection with the INTERNATIONAL CONFERENCE ON THE MOLECULAR BIOLOGY AND BIOTECHNOLOGY OF CILIATES AND ANAEROBIC PROTOZOA (Abstracts 75–78)

## 75

Spathidiid Ciliates—A Re-evaluation of Their Morphological Diversity. K. XU and W. FOISSNER, University of Salzburg, Institute for Zoology, A-5020 Salzburg, Austria.

The spathidiids are a large assemblage with families, genera and species often differing by rather inconspicuous features. Several authors consider spathidiids as indeterminable and suppose synonymy of many species. We re-evaluated their morphological diversity by investigating species from soils worldwide, using live observation, protargol impregnation, scanning electronic microscopy, and morphometry. The data suggest the following main features for distinguishing genera: the somatic ciliary pattern (*Spathidium*-, *Protospathidium*-, *Arcuospathidium*-, and *Epispathidium*-type); the structure (number of rows increased in an undescribed genus; isomorphic, heteromorphic) and location (dorsal; lateral in *Cultellothrix* and an undescribed genus) of the dorsal brush; the shape of the circumoral kinety (open in *Apertospathula*) and oral bulge (discoidal in *Semispathidium*); and the number and configuration of the contractile vacuoles (≥ 5 vacuoles each having several excretory pores in *Supraspathidium*). More recently, a striking feature, viz., oralized somatic monokinetids were discovered in two genera (*Apospathidium* and an undescribed genus), further increasing spathidiid diversity. Obviously, spathidiids are a melting-pot of rather different genera, whose relationship is, however, very difficult to analyse. Likewise, there are indeed many *Spathidium* (s.l.) species becoming, however, obvious only on very careful investigation. Much of the spathidiid diversity is still undescribed.

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## 76

Redescription and Phylogenetic Relationships of the Supposedly Well-known Marine Planktonic Ciliate *Laboea strobila* Lohmann, 1908 (Ciliophora, Oligotrichia), with Notes on its Cultivation, Ecology, and Ontogenesis. S. AGATHA*, M. C. STRÜDER-KYPKE** and A. BERAN***, *Institute for Zoology, University of Salzburg, A-5020 Salzburg, Austria, **Department of Zoology, University of Guelph, Guelph, ON, N1G 2W1, Canada, ***Laboratorio di Biologia Marina, I-34010 S. Croce/Trieste, Italy.

Laboea strobila Lohmann, 1908 is a conspicuous oligotrich ciliate in the marine plankton. It is the sole species in the genus Laboea and has been reported from various locations. In order to compare different populations collected from the Mediterranean Sea, North Sea, and Irish Sea, we investigated the morphology and ontogenesis using live observation, protargol im-