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Temporal succession and diversty of biofilm cilitates in a RBC plant. B. PÉREZ-UZ, M. MARTÍN-CERECEDA, S. SERRANO and A. GUINEA, Dept. Microbiología III. Facultad CC. Biológicas. Universidad Complutense de Madrid. Spain.

Biofilms of a wastewater treatment plant by rotating biological contactors (Boadilla, Madrid-Spain) have been studied during a year. Sampling was carried out in three stages along the biological treatment. Spatial and temporal microbial succession in this variable physical-chemical background was explored to prove the differential distribution of the protists populations. Protists communities of these biofilms consisted mainly of ciliates and diatoms. Diatoms represented a high proportion along the system, reaching in the last stages of the treatment over 50% of protist abundance. Therefore, they were an important biological factor in the biofilm cohesion, in contrast with that observed in other RBC plants studied. A low ciliate diversity and abundance in the first stage sampled of the biological system was found; only species of the genus *Epistylis* sp. and *Vorticella convallaria* appeared in high numbers along the whole year. A significant increment on both diversity and abundance of ciliates was observed in the intermediate stage sampled, where peritrich ciliates showed a clear seasonal succession. *Opercularia coarctata* appeared in spring and was substituted by *Epistylis* species during autumn and summer, and then in winter by *V. convallaria.* The last stage showed a decrease of peritrich ciliates and only *Zoothamnium procerius* appeared with a high density along the year. A seasonal succession of non-sessile groups was observed in this stage; populations of *Aspidisca lynceus* and *A. cicada* appeared in spring and were replaced by *Litonotus lamella, Dexiotricha* sp. and *Uronema nigricans* during summer and autumn while in winter the dominant species was *Trochilia minuta.* These results suggest that seasonal species succession allow the same functional groups to occupy the same trophic niche in the biofilms.

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Dissecting the sexual cycle of *Trypanosoma brucei.* A. MacLEOD*, A. TWEEDIE*, S. TAYLOR**, S. McLELLAN**, C.M.R. TURNER** and A. TAIT*, *Wellcome Centre for Molecular Parasitology, Anderson College, University of Glasgow, 56, Dumbarton Road, Glasgow, G11 6NU, **Division of Infection & Immunity, IBLS, Joseph Black Building, University of Glasgow, Glasgow, G12 8QQ.

Genetic exchange is a fundamental biological process, which results in the generation of diversity. The process by which *T. brucei* undergoes genetic exchange has been the subject of debate for some time with several different models of mating being proposed. Here we describe the genetic analysis of a large number of progeny clones from three laboratory crosses and present evidence that genetic exchange in *T. brucei* is Mendelian. We demonstrate sexual recombination (in agreement with Mendel's first law), independent segregation (Mendel's second law), crossing over between homologous chromosomes and a low frequency of triploidy and trisomy. The evidence unequivocally supports a standard diploid meiotic Mendelian system of genetic exchange.

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Virulence of Oocysts of the Czech *Toxoplasma gondii* Isolates. E. BARTOVA*, K. SEDLAK** and I. LITERAK, *Department of Biology and Wildlife Diseases, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic, **Department of Virology, State Veterinary Institute, Prague, Czech Republic.

Virulence of oocysts of the seven Czech *Toxoplasma gondii* isolates was tested on the base of biological and genetic characteristics. The oocysts from individual isolates were obtained by experimental infection of cats with the 300–600 tissue cysts of *T. gondii. T. gondii* was isolated in 1994–1995 from dog (isolate K1), cats (isolates K2, K21 and K25) and from rabbits (isolates K7, K9 and K19). The cats shed oocysts in feces with prepatent periods of 3–5 days post infection (DPI). The patent periods were 7–18 days. The number of oocysts shed varied from $0.94–47 \times 10^6$ and most of the oocysts were shed usually at 5–6 DPI (in isolate K7 it was at 17 DPI) with $0.66–39 \times 10^6$ oocysts in the daily sample of excrements. The cats stopped their oocyst excretion at 11–22 DPI. The sporulated oocysts were used for preparing of the infection doses of $1–1 \times 10^5$ oocysts, that were used for oral infection of 10 mice. After infection, the mortality of infected mice was tested. Isolated DNA from four *T. gondii* isolates was used in polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) for amplification of gene ROP1 and restriction of the product of amplification by restriction endonuclease *Dde*I. Based on biological characteristics, all seven isolates were included in a group of "avirulent" strains. Avirulent strains are in literature usually characterized by LD100 > 103 tachyzoits and presence of the tissue cysts in brain during chronic infection in contrast to "virulent" strains with LD100 < 10 tachyzoits and presence of ascites during acute and lethal infection. Nevertheless, among isolates there was difference in their virulence. The most virulent isolate was isolate K1, followed by isolate K2 and isolates K7 and K21. The isolates K9, K19 and K25 were the least virulent, neither dose of 10^5 oocysts represented lethal dose for mice. In PCR/RFLP, two isolates K9 and K19 showed common pattern of avirulent strain. Isolates K21 and K25 showed patterns of both, avirulent and virulent strains.

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The Role of *Leishmania* Surface Metaloprotease gp63 in Sand Fly Vector. M. HAJMOVA*, P. VOLF*, B.K. KOLLI** and K.-P. CHANG**, *Charles University, Prague, Czech Republic, **FUHS/Chicago Medical School, N Chicago, IL, USA.

The zinc-protease (gp63) of promastigotes was found to play a role in the sand fly part of *Leishmania* life cycle. We demonstrated this by using transfectants of a *L. amazonensis* clone whose gp63 was up- and down-regulated by directional cloning into P6.5 for sense- and anti-sense transcription (Chen et al. 2000, *Inf. Immun.* **68**:80–86). *Lutzomyia longipalpis* females were infected by membrane feeding (106 promastigotes/ml). Midgut infections were found to differ between the sense- and antisense-transfectants 2 days postfeeding. The sense transfectants overexpressing gp63 were found similar to the controls (P6.5 with the vector alone): both produced infection at high

rates (~90–100%) and at a high intensity (moderate to heavy infection in >70% of the infected females). In contrast, the antisense transfectants with gp63 down-regulated produced infection at a lower rate (~70%) and at a very low intensity (moderate to heavy infection in 20% of the infected females). On day 9 postfeeding all three groups of transfectants produced similar infection rate of ~50% with comparable parasite loads and colonization of the stomodeal valve. The results obtained appear to indicate that down-regulation of gp63 does not prevent parasite growth in sand flies in late stage infections but may play a role at the early stage of midgut infections during bloodmeal digestion.

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The Polarity of Anagenesis of Motility Apparatus and Repeated Switches of Life Strategies in Trichomonad Flagellates. V. HAMPL, I. CEPICKA, J. KULDA, J. FLEGR and J. TACHEZY, Department of Parasitology, Charles University, Vinicna 7, 128 43 Prague 2, Czech Republic.

Trichomonad flagellates, living mostly as harmless intestinal commensals include also some pathogenic species and three free-living genera. A common cell structure of typical trichomonad genera is the undulating membrane the main motility organelle, which is usually underlied by a supportive cytoskeletal fibre the costa. However, several parasitic and all free-living genera of trichomonads lack costa or both costa and undulating membrane. In the morphology-based concept of trichomonad evolution these genera have been considered as ancestral. In conrast, in the 16S rRNA phylogeny these taxa do not form a monophyletic group nor occupy ancestral positions. Here we present a more detailed phylogenetic analysis of the order Trichomonadida based on the sequences of 16S rRNA including 18 taxa that lack undulating membrane and costa. These taxa formed four groups in the tree, none of which was in the ancestral position. This suggests that the undulating membrane and costa were secondarily lost during evolution rather than that their absence represents a primitive state. Three of these groups are exclusively parasitic, while one includes both parasitic and free-living species. Inside the latter group the free-living genera (*Pseudotrichomonas, Ditrichomonas, Monotrichomonas*) together with cattle parasites (*Monocercomonas ruminantium*) formed a robust branch distinct from four other parasitic taxa that branched in this clade. The cattle parasites were placed inside this free-living branch very close to the species *Pseudotrichomonas keilini* (97.3% similarity). The distal position of free-living taxa in the trichomonad tree indicates a possible reversal from a parasitic to a free-living way of life. In contrary, the most parsimonious explanation for the position of cattle parasites inside the free-living trichomonad branch is their recent switch from free-living to parasitic life strategy.

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Haematology and Leucocytozoonosis of Great Tits (*Parus major* L.). K. HAUPTMANOVA, I. LITERAK and E. BARTOVA, Department of Biology and Wildlife Diseases, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic.

We performed an examination of 81 great tits (*Parus major*) from Brno, Czech Republic, in winter 2000/2001. An injured great tit having its leg amputated was also tested. The blood was tested for the incidence of blood parasites and haematology. In three healthy birds (3.9%, n = 77) we distinguished the blood parasites of *Leucocytozoon* genus, in one case (1.3%, n = 77) *Haemoproteus* sp., and in one case (1.3%, n = 77) microfilariae (in all cases 1 parasite per 10^5 erythrocytes). *Plasmodium* spp. or *Trypanosoma* spp. were not found. The injured great tit suffered from the infection with blood parasite *Leucocytozoon dubreuili*. The examination of 80 healthy birds gave us the following parameters: $5.89 \pm 1.36 \times 10^6$/l erythrocytes, haemoglobin content 173.2 ± 29.9 g/l, packed cells volume 45 ± 4%, and $4.06 \pm 2.40 \times 10^3/\mu$l leucocytes (68.5% lymphocytes, 19.6% heterophils, 5.6% eosinophils, 5.6% basophils and 1.0% monocytes). In the blood of the injured great tit, we found anaemia and leucocytosis. We assume that the enfeeblement of organism by the trauma resulted in immunity decrease allowing the reactivation of latent infection *L. dubreuili*.

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Impact of High and Low Temperatures on Viability of Chicken Coccidian Oocysts. J. HOLKOVA and P. BEDRNIK, BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs, Department of Protozoology, 254 49, Jilove u Prahy, Czech Republic.

Chicken coccidia become a permanent problem in poultry farming because of their extraordinary survival abilities. The aim of the presented study was to examine devitalization effects of high and low temperatures on sporulated oocysts of chicken Eimeria species. The oocysts of *Eimeria acervulina, E. maxima* and *E. tenella* (LIVACOX® T) were exposed to high temperatures +50°C (for 15, 30, 45, 60, 90 minutes), +55°C, +60°C and +80°C for 5 minutes; and to low temperatures −12°C, −22°C, −80°C (for 60 minutes) and −22°C, −80°C for 24 hours. Treated oocysts were inoculated via crop tube (dose 10.000 oocysts of each species/chicken) or via drinking water (dose 11.150 oocysts of each species/chicken) to ROSS 308 chickens aged 10–14 days. The chickens were raised in isolators (on litter) to avoid external contamination and fed a broiler starter diet free of anticoccidials. From the 5th to 8th day p.i. discharging of oocysts in feces (OPG) was followed and their species diagnostics was performed. The experiments proved a great sensitivity of all three tested *Eimeria* species to the high temperatures. The lethal effect was demonstrated already after exposure to +50°C for 30 minutes. The coccidian oocysts remained viable when exposed to temperatures up to −12°C for 60 minutes, however, the exposure to −22°C for 60 minutes resulted in their devitalization.

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The Sporulation of Oocysts of Fowl's Coccidia and Possibilities to Influence This Process. M. HORTVIKOVA and P. BEDRNIK, BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs, Department of Protozoology, 254 49, Jilove u Prahy, Czech Republic.

The subject of this work was to monitor factors influencing the oocyst's sporulation; namely: concentration of potassium dichromate ($K_2Cr_2O_7$) and the presence of atmospheric oxygen. In this study oocysts of *Eimeria maxima, E. tenella* and *E. acervulina* were used. The sporulation percentage was counted after 48 hours of sporulation at 25–26°C, oocysts were removed by centrifugation and the $K_2Cr_2O_7$-supernatant was reused. We focused on determination of the lowest concentration of $K_2Cr_2O_7$that still enables oocyst sporulation and necessity of presence of atmospheric oxygen. Obtained results proved that the lowest possible concentration of $K_2Cr_2O_7$ for sporulation is 0.5% for all three tested *Eimeria* species and that it is possible to reuse it for 3, max. 4 sporulations of tested coccidian oocysts.

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Effect of Nisin and Monensin on Rumen Ciliates and Fermentation in Artificial Rumen. S. KISIDAYOVA, A. LAUKOVA and D. JALC, Institute of Animal Physiology, Slovak Academy of Sciences, Kosice, Slovakia.

The objective of this study was to investigate the effect of nisin (as Nisaplin, 2.5% content of nisin) and monensin on population of rumen ciliate protozoa and rumen fermentation of diets containing hay and barley (80:20%) in artificial rumen (the Rusitec system). The Rusitec system consisted of four fermentation vessels (V1, V2, V3, V4): V1 was without additives (control), V2 received daily 2 mg of nisin, V3 involved 5 mg of monensin and V4 combination of 2 mg of nisin with 5 mg of monensin. After an adaptation period (7 days), the fermentation parameters and changes in ciliate population were determined for six consecutive days. Compared to control diet, the addition of nisin resulted in an increase ($P < 0.05$) of hemicellulose degradation, acetate and propionate (mmol/day) production and energetic efficiency of VFA (E), while butyrate production decreased. Nisin had no effect on dry matter (DM), organic matter (OM), cellulose and detergent fiber degradability, production of total gas, methane and efficiency of microbial synthesis. The addition of monensin resulted in a decrease of DM, OM ($P < 0.05$), cellulose, hemicellulose, detergent fiber degradability ($P < 0.001$), total gas, methane and ammonia nitrogen (NH3-N) production. Monensin also significantly decreased acetate, butyrate, L-lactate (mmol/day) production and it increased propionate production ($P > 0.001$) and efficiency of microbial synthesis. The combined effect of nisin and monensin in V4 was similar to the effect of monensin in V3 compared to control. Then, the effect of additive monensin was dominant over nisin. Major protozoan ciliate groups in RUSITEC were *Entodinium* spp. and *Dasytricha ruminantium*. The supplementation of nisin significantly increased the population of both major groups of ciliates. The supplementation of monensin significantly decreased the population of the two ciliate groups. The combined effect of nisin and monensin was similar to the effect of monensin compared to the control. In conclusion, our results indicate that nisin was less effective as monensin on some fermentation parameters (important for the improvement of the efficiency of utilization of the diet by ruminants) and stimulatory to ciliate population in artificial rumen.

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Eimeria tenella: Significance of Level of Litter Contamination with Oocysts and Its Humidity for Outbreak of Coccidiosis. G. MATEJU* and P. BEDRNIK**, *Czech Agricultural University, Prague, **BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs, Jilove u Prahy, Czech Republic.

The purpose of this study was to determine a level of litter contamination with oocysts of *Eimeria tenella* evocating outbreak of clinical coccidiosis under conditions of different litter humidity. Each experimental group consisted of ten 14-day-old Ross 308 chickens placed in an isolator with litter of wood shavings. The litter was moistened and contaminated with *E. tenella* sporulated oocysts before housing of chickens according to design of each trial. In the first trial each gram of litter was contaminated with either 10^4 or 10^5 of oocysts, but not moistened. In further trials the level of contamination of the litter with oocysts was decreased gradually into 2.5×10^3 oocysts/g of litter and the relative humidity (RH) of the litter was gradually increased up to 60%. The course of coccidiosis was evaluated by findings of blood in droppings, mortality and scoring of coccidian lesions caused by *E. tenella* of surviving chickens. In the first trial with not moistened litter, only chickens placed on the litter with the dose of 10^5 oocysts/g suffered from clinical coccidiosis. In further trials when RH of litter was increased to 45%, the dose 7.5×10^3 oocysts/g of litter caused outbreak of clinical coccidiosis, but no mortality. When RH of litter was 60% and contamination dose 10^4 oocysts/g, 6 chickens in this group died from coccidiosis. The susceptibility of chickens to coccidiosis was highly influenced by the increased humidity of litter.

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Endogenous Development of Rabbit Coccidium *Eimeria flavescens*. M. PAKANDL* and F. CERNIK**, *Institute of Parasitology, Academy of Sciences of the Czech Republic, Ceske Budejovice, **BIOPHARM, Research Institute of Biopharmacy and Veterinary Drugs, Jilove u Prahy, Czech Republic.

The endogenous cycle of *Eimeria flavescens* was studied in SPF rabbits orally inoculated with graded doses of oocysts (from 5×10^6 to see initial stages of the development to 5×10^4 in order to study the last merogony and gamogony). The samples taken from the intestine were processed for histology and transmission electron microscopy. We noted a total of five asexual generations and, like in the other rabbit species whose development has been studied by electron microscopy, two types of meronts and merozoites in each generation. Type A gave rise to a smaller number of fat polynucleated merozoites in which daughter merozoites were formed by endomerogony, while in the type B meronts slender uninucleated merozoites arose from ectomerogony. The first generation meronts were found in the crypts of the duodenum and jejunum, whereas the three following generations developed in superficial epithelium of the large intestine (cecum, vermiform appendix and colon) and the last merogony as well as gamogony took place in crypts of the large intestine. The sporozoites were first seen as late as 64 hours past inoculation (p.i.) in the lamina propria in the vicinity of crypts and the first meronts were found 80 h p.i. in the epithelium of the crypts. Such a long migration phase is remarkable, but we are sure that the beginning of asexual multiplication was recorded since we observed the sporozoites. Although the apical complex and other organelles typical for sporozoites were conserved, a nuclear division and formation of daughter merozoites were noted within the sporozoites. An unusually large number of nuclei, up to nine in a plane of section, were seen in the fifth generation merozoites of the type A. On the level of electron microscopy, this study is the first to give a description of the endogenous development of *E. flavescens* and the only to show that endogenous development is not completed in the same intestinal segment. The appearance of two types of meronts was not so far demonstrated in any asexual generation of this coccidium.

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Sand Fly Salivary Proteins and Antibody Response of Bitten Hosts. I. ROHOUSOVA, P. CERNA, L. MIKES and P. VOLF, Department of Parasitology, Charles University, Prague, Czech Republic.

Sand fly saliva modulates haemostasis and immunity of the host having an enhancing effect on *Leishmania* infection. Hyaluronidase, enzyme we characterized in six sand fly species, may participate in all these effects. Sand fly hyluronidases cleave hyaluronic acid and chondroitin-sulfates A and C, essential components of mammalian extracellular matrix and thus

increase the permeability of host tissue for other pharmacological compounds of saliva. In *Phlebotomus* (*Larroussius*) *perniciosus* and *Lutzomyia longipalpis* the active molecule is a dimmer forming intermolecular disulfide bonds, in members of subgenera *Phlebotomus* and *Adlerius* it consists of a single polypeptide chain. In bitten hosts, sand fly saliva stimulates an immune response that may neutralise enhancing effect of saliva on *Leishmania* infections. ELISA revealed high titres of specific antibodies against *P. papatasi* and *P. sergenti* in humans from Sanliurfa, Turkey, where these two sand fly species represent more than 99% of sand fly population. Dot-blots and immunoblots using sera of animals experimentally bitten by various sand fly species showed striking differences in the salivary antigens. Four to nine antigenically distinct peptide bands were detected in each species, no cross-reactivity was found between *P. papatasi* and other species tested. In conclusion, saliva of various sand fly species significantly differs in enzymatic and antigenic properties.

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Toxoplasmosis in Antelopes: Finding of Tissue Cysts in Liver in Saiga Antelope (*Saiga tatarica*). K. SEDLAK, Department of virology, State Veterinary Institute Prague, Sidlistni 24, 165 03, Prague, Czech Republic.

Fatal toxoplasmosis was diagnosed in one captive Saiga antelope (*Saiga tatarica*) from a Zoo in the Czech Republic. The adult Saiga antelope died suddenly, without any apparent clinical signs. *Toxoplasma gondii* was found in liver, lungs and spleen. Large number of tissue cysts were showed in liver. Toxoplasmic hepatitis and pneumonia were considered as probable primary cause of death, however, *Mullerius capillaries* and *Pasteurella multocida* were also detected in lungs. Retrospectively, a total number of 181 serum samples collected from bovids in captivity in 9 Zoos in the Czech Republic and Slovakia from 1999–2001 were examined for antibodies to *T. gondii* by indirect fluorescence antibody test. *T. gondii* antibodies were demonstrated in 27.6% (50 positive/181 examined). Among them 18 species of exotic bovids were positive, other 10 species (included Saiga antelopes) were negative.

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Molecular Characterization of the Iron-Hydrogenase Gene Homologue from *Cryptosporidium parvum.* F. STEJSKAL*, J.R. SLAPETA**, V. CTRNACTA* and J.S. KEITHLY**, *Department of Tropical Medicine, 1st Faculty of Medicine, Charles University, 128 00 Prague, Czech Republic, **Wadsworth Center, New York State Department of Health, Albany, NY.

Hydrogenases catalyze the reversible oxidation of molecular hydrogen and play a central role in microbial energy metabolism. Fe-hydrogenases represent a phylogenetically distinct class found and characterized mainly in anaerobic prokaryotes and protists. Only recently, there have been identified Fe-hydrogenase gene homologues in other unicellular and multicellular eukaryotes. We have cloned and sequenced *Cryptosporidium parvum* Fe-hydrogenase (CpHdg) gene homologue. CpHdg is an intronless gene of 1,680 bp encoding a protein of 560 amino acids (aa) with a calculated Mr of 63.1 kD. The Southern blot analysis indicates that there is a single-copy gene in the *C. parvum* genome. CpHdg is homologous to other Fe-hydrogenases: 1) contains one highly conserved iron-sulfur binding site at the N-terminus; 2) contains all conserved aa residues proposed for catalytic function within a H-cluster; 3) shares 42–53% aa sequence similarity with *Giardia intestinalis, Trichomonas vaginalis,* yeast, mammalian and plant Fe-hydrogenases. In the phylogenetic analysis CpHdg grouped together with fungal, kinetoplastid, animal and plant Fe-hydrogenase homologues. (Supported by NIH-FIRCA 1R03 TW01507-01 to FS and JSK.)

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Experimental Transmission of *Leishmania tropica* to Hamsters and Mice by the Bite of *Phlebotomus sergenti.* M. SVOBODOVA and J. VOTYPKA, Department of Parasitology, Charles University, Vinicna 7, 128 43 Prague 2, Czech Republic.

The sand fly *Phlebotomus sergenti* is considered to be a natural vector of *Leishmania tropica.* However, the ability of *P. sergenti* to transmit *L. tropica* by bite has not been proven experimentally yet. We demonstrate transmission of *L. tropica* to albino golden hamsters (*Mesocricetus auratus*) and BALB/c mice by the bite of *P. sergenti.* Sand flies and *L. tropica* both originate from an anthroponotic cutaneous leishmaniasis focus in Urfa, Turkey. Sand fly females from a laboratory colony were infected by feeding on lesions of *L. tropica* needle-inoculated hamsters or mice. Gravid females were allowed to feed again 9–15 days after the infective feed. Three hamsters and four mice were used. At the second feed some infected sand fly females took a full blood meal, while others only a partial one. The blocked stomodeal valve or the presence of the eggs in the abdomen might both influence the volume of the second feed. However, some females failed to feed at all. In two albino hamsters swelling developed 1 month after the infective feed, and *Leishmania* were reisolated from these sites. Another hamster did not develop any cutaneous changes, but the feeding site (fore foot) and the adjacent ear were PCR positive 1 year after infective feeding. In all 4 BALB/c mice swelling developed after 4–6 months. The lesions did not ulcerate. Experimental transmission of the parasite confirms that *P. sergenti* is a natural vector of *L. tropica.* Transmissibility to inbred mice enables the use of a better-defined model host.

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Pyruvate decarboxylase of Metronidazole-Resistant *Tritrichomonas foetus.* R. SUTAK, J. KULDA, J. TACHEZY and I. HRDY, Department of Parasitology, Faculty of Science, Charles University in Prague, Prague 2, Czech Republic.

Tritrichomonas foetus, the agent of a sexually transmitted disease of cattle, can develop resistance against metronidazole and related 5-nitroimidazole drugs. It has been found that the development of resistance is underlined by defects in oxygen-scavenging system and progressive downregulation of hydrogenosomal proteins involved in drug activation. This process results in dramatic rearrangement of carbon flow in the trichomonad cell. Most of the hydrogenosomal enzymes that constitute the ATP-generating pathway are lost and production of acetate and hydrogen by the organelle is ceased. Instead, the rate of cytoplasmic glycolysis accelerates and ethanol becomes a dominant metabolic end product. Ethanol production is catalysed by pyruvate decarboxylase (PDC) and alcohol dehydrogenase. The activity of the former enzyme, negligible in susceptible tichomonads, is substantially upregulated in the resistant organisms. Since PDC is not present in mammalian cells, it appears to be a suitable target for chemotherapeutic intervention against drug-resistant parasites. In order to investigate this possibility, we purified the PDC from *T. foetus* cytosol by conventional liquid chromatography methods. We found that the enzyme is a TPP-dependent tetrameric protein with subunit size of 60 kDa as are most other PDCs. The pH optimum of the enzyme was 5.75–6.5, the Km for pyruvate 0.62 mM. Ke-

tomalonate, indolpyruvate, glyoxylate and omeprazole inhibited the enzyme, with glyoxylate and omeprazole being most effective. Omeprazole, an approved drug against ulcer disease, showed inhibitory effect against metronidazole-resistant trichomonads in vitro, while metronidazole-sensitive strain was not affected.

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Mitosis in *Giardia intestinalis.* P. TESAROVA*, J. NEBESAROVA**, J. REISCHIG*** and E. NOHYNKOVA*, *Department of Tropical Medicine, 1st Faculty of Medicine, Charles University, Prague, **Laboratory for Electron Microscopy, Institute of Parasitology, Academy of Science of the Czech Republic, Ceske Budejovice, ***Department of Biology, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic.

Giardia intestinalis is a unicellular bi-nucleated eukaryote causing enteric disease in humans and animals worldwide. This parasitic flagellate is extraordinary in that it possesses two structurally and, possibly, functionally identical nuclei within the cell of a vegetative trophozoite stage. The *Giardia* cell multiplies by binary fission, mitosis being the exclusive process of karyokinesis of the nuclei. However, due to a very short duration of the process and a lack of a method for synchronizing *Giardia* cells in vitro, data on the course of the mitosis are scarce and controversial. Here we present for the first time complete sequence of the process based on light and electron microscopy observations of *Giardia* population enriched with mitotic cells. During cell division, both nuclei divide in parallel. Two bipolar mitotic spindles are assembled, located between separated kinetosomal complexes of flagella perpendicularly to longitudinal body axis. Spindle poles are acentriolar with small ring-like structures to which spindle microtubules converge. Nuclear membrane remains mostly intact except of small fenestrae formed at each pole of the dividing nucleus. During meta- and anaphase, each spindle is composed of a one-layer extranuclear corset of about 50 microtubules plus 10 to 20 microtubules penetrating nuclear membrane through the polar fenestrae thus entering the nucleus. In this respect, it closely resembles mitotic spindle of *Hexamita.* While gamma-tubulin was not identified at spindle poles at any phase of the mitosis in *Giardia,* no data are available about other centrosome-associated proteins. Using a nomenclature of Raikow (1994), mitosis in *Giardia* represents an unusual type of semi-open orthomitosis with an extranuclear spindle.

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Seroprevalences of Antibodies Against *Neospora caninum* in Cattle and Dogs in the Czech Republic. P. VACLAVEK*,**, B. KOUDELA*,**, K. SEDLAK*** and R. SEBESTA****, *Department of Parasitology, University of Veterinary and Pharmaceutical Sciences, Palackeho 1-3, 612 42 Brno, **Institute of Parasitology Academy of Sciences of the Czech Republic, Branisovská 31, 370 05 Ceske Budejovice, ***State Veterinary Institute, Laboratory of Virology and Serology, Sidlistni ul. 136/24, 165 03 Praha 6—Lysolaje, ****Veterinary Base Grabstejn, 463 34 Hradek n. Nisou, Czech Republic.

A serological surveys for antibodies against *Neospora caninum* in cattle and dogs in the Czech Republic were carried out. The seroprevalence of *N. caninum* was evaluated in 430 serum samples of aborting cattle. Then, three farms (A, B, C) with confirmed occurrence of *N. caninum* in aborting cattle were selected for estimation of seroprevalence in normally calving cattle. The sera were analyzed for *N. caninum* specific antibodies by indirect fluorescent antibody test (IFAT) and commercial enzyme-linked immunosorbent assay (IDEXX ELISA). The analysis of serum samples demonstrated antibodies against *N. caninum* in 41 (9.5%) of 430 aborting cows. In serum samples from 306 normally calving diary cattle from chosen three farms (A, B, C) were detected *N. caninum* antibodies in 4 (3.2%) of 126 tested cattle from farm A, 5 (3.8%) of 132 from farm B and 0 (0%) of 48 from farm C. The results reveal a presence of specific antibodies in herds of diary cows and it is indicating that both aborting and non-aborting cows were exposed to *N. caninum* and neosporosis should be considered in differential diagnosis of bovine abortion in the Czech Republic. The antibodies to *N. caninum* were IFAT-assayed in sera of 470 military dogs. The seroprevalence was 5.9% (28/470) and none of serological positive dog was apparently suffering clinical neosporosis. The antibody titers of dogs were 1:50 (16 dogs), 1:100 (3 dogs), 1:200 (5 dogs), and greater than or equal to 1:400 (4 dogs). Our results suggest that the infection by *N. caninum* is present in dogs in the Czech Republic and should be considered in the clinical diagnosis of dogs presenting neuromuscular disorders.

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Bacterial-Type NADH : Ferredoxin Oxidoreductase Mediates Alternative Electron Transport in *Trichomonas vaginalis* Hydrogenosomes. J. VASAK, R. SUTAK, P. DOLEZAL, J. TACHEZY and I. HRDY, Department of Parasitology, Faculty of Science, Charles University, Prague, Czech Republic.

Hydrogenosomes of trichomonads utilize both pyruvate and malate in catabolic reactions that result in the substrate-level synthesis of ATP and production of molecular hydrogen. When pyruvate is the oxidized substrate, the released electrons pass through an electrontransport chain that consists of an electron-generating enzyme pyruvate : ferredoxin oxidoreductase, electron-transporting protein ferredoxin and terminal oxidoreducatase hydrogenase that reduces protons to hydrogen. Alternatively, when malate serves as substrate, it is first oxidatively decarboxylated to pyruvate and CO2 by NAD-specific malic enzyme. Resulting NADH must be reoxidized and this is postulated to happen by action of NADH : ferredoxin oxidoreductase (NFOR) that transfers the electrons to ferredoxin. Activity of NFOR could be measured as NADH-dependent reduction of artificial acceptor methyl viologen. We have purified, characterized and cloned this enzyme. It is a 45 kDa monomeric flavoprotein that reduces trichomonad ferredoxin and number of other acceptors including oxygen, FAD, FMN, ferricyanide, benzyl viologen and tetrazolium salts. Amino-terminal part of translated sequence contains a short extension that is missing from purified protein and that strongly resembles hydrogenosomal targeting signal from other hydrogenosomal proteins. BLAST search identified a number of homologous sequences, mostly archaebacterial and eubacterial flavoproteins without defined function. The two characterized homologs were rubredoxin-oxygen oxidoreductase from *Desulfovibrio gigas* and electron-transporting flavoprotein from the archaeon *Methanobacterium thermoautotrophicum.* Homologous open reading frames without assigned function were also found in {Entamoeba histolytica} and *Giardia intestinalis* genome project databases. The trichomonad protein is dissimilar to NAD(P) : ferredoxin oxidoreductases from chloroplasts and mitochondria. It is the first protein of this type to be characterized from a eukaryotic organism.

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Trichomonad affinities of *Cochlosoma* confirmed by sequence analysis of 16S rRNA gene. M. VRLIK*, J. KULDA*,

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Protozoan flagellates of the genus *Cochlosoma* are intestinal parasites of birds, bats and shrews. The organisms are equipped with 6 flagella, undulating membrane, axostyle and a conspicuous adhesive disk that serves for the parasite attachment to the intestinal mucosa. Taxonomic position of *Cochlosoma* has been uncertain, however, recent ultrastructural study of the type species *Cochlosoma anatis* disclosed apparent homologies with trichomonads. Here we report results of phylogenetic analysis based on sequences of SSu rRNA gene involving 27 species of different genera of the order Trichomonadida Kirby, 1947. A full length *Cochlosoma* gene for 16S rRNA, 1508 bp in lenght, was isolated from washed *Cochlosoma* trophozoites obtained from experimentally infected ducklings. Alignments were constructed with the aid of Clustal X program and edited in MUST; 1367 positions were included into analysis. The data were evaluated by means of Phylip and PAUP* programs, using Neigborjoining, Maximum parsimony and Maximum likehood methods for tree construction. The results confirmed unequivocally pertinence of *Cochlosoma* among Trichomonadida, with apparent affinity to the Trichomonadinae branch of the trichomonad lineage. Members of the Trichomonadinae subfamily assumed consistently position of a sister group to *Cochlosoma* in the constructed trees. Examination of *Cochlosoma anatis* by transmission electron microscopy provided additional support for a close relationship of *Cochlosoma* with Trichomonadinae. Presence of B-type costa and lamelliform undulating membrane, common to Trichomonadinae genera was demonstrated, while mastigont associated features such as comb or suprakinetosomal body characteristic for Tritrichomonadinae, the other major trichomonad branch, were absent. Still, *Cochlosoma* showed numerous unique characters and further studies are required for a sound assessment of its taxonomic rank within the Trichomonadida.

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A Biocomplexity Model of Protistan Communities in Soils and Marsh Substrata with an Emphasis on the Sarcodines. O.R. ANDERSON, Biology, Lamont-Doherty Earth Observatory of Columbia University, Palisades, NY.

Biocomplexity theory is increasingly important in understanding ecosystem dynamics as we realize that the interactions among subunits in a multi-component system often produce elaborate states that are not easily explained in terms of the individual parts of the system. However, devising scientifically sound models that permit reproducible applications in natural settings can be challenging. A Euclidean geometric model of protistan biocomplexity is presented based on three quantitative biotic dimensions (indices) for small samples of substrata (0.01 g): (1) Mean density of protists per 0.01 g, (2) uniqueness of morphospecies distribution among the 0.01 g samples, and (3) patchiness of the distribution of protists across the 0.01 g samples. These three indices are mapped into a three-dimensional space model and the geometric distance of the sample point from the origin in Euclidean space (based on the three dimensions) is used as a general index of biocomplexity. Results of applying the model to a range of terrestrial and marsh communities is presented illustrating the usefulness of the model in discriminating community biocomplexity across diverse environments.

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Influence of Suspended Solids and Host Diversity on Dinoflagellate Parasitism. T.-N. ARMSTRONG* and D. W. COATS**, *University of Maryland College Park, College Park, MD, **Smithsonian Environmental Research Center, Edgewater, MD.

The Chesapeake Bay is the largest estuary in the United States and is located in Maryland and Virginia. The Bay is also home to many planktonic organisms including the red-tide dinoflagellate *Akashiwo sanguinea* and its dinoflagellate parasite *Amoebophrya* sp. Since the 1960's there has been increasing nutrients and sediments in the Bay that have been negatively affecting the natural balance of the ecosystem. While blooms of *A. sanguinea* have been linked to fish kills in Korea and Louisiana, no harmful effects have been noted in Chesapeake Bay. A natural control of *A. sanguinea* in the Chesapeake is the parasite *Amoebophrya* sp. The ability of *Amoebophrya* to infect and control *A. sanguinea,* however, depends on many factors. For example, parasite dispersal stages, dinospores, may adhere to particulates and fail to establish infections, as is know to occur in viruses. In addition, laboratory studies indicate that the parasite can infect inappropriate host species and thus fail to complete its life cycle. In this study, we investigated whether suspended solids or the presence of non-host species influences the success of *Amoebophrya.* We found that suspended solids do not alter parasite prevalence or parasite load in *A. sanguinea.* However, the presence of another species, the toxic dinoflagellate *Karlodinium micrum,* reduced parasite success in *A. sanguinea.* The negative effect appeared to result from dissolved substance released by *K. micrum,* rather than mistaken infection of the inappropriate host. Results suggest that suspended solids have little impact on the prevalence of *Amoebophrya.* They also indicate that phytoplankton species composition may limit the spread of infections, with epidemic outbreak that lead to the decline of host populations more likely to occur in monospecific blooms.

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A SEC7-Related Protein in *Tetrahymena.* A. J. BELL, A. AWAN and P. SATIR, Albert Einstein College of Medicine, Bronx, NY.

SEC7 was first identified in mutants of *Saccharomyces cerevisiae* that accumulated golgi stacks and cisternae when incubated at 37°C in low glucose medium. Since then, Sec7-related proteins have been found in a variety of organisms including *Homo sapiens, Arabidopsis* and *Paramecium.* In addition to their role in vesicular transport, many of the Sec7-related proteins have been shown to function as guanine-nucleotide exchange factors (GEF) for small G-proteins such as ARF. *Paramecium* SEC7 (PSEC7) has been cloned and shown to be upregulated upon deciliation, implicating a role of Psec7 in ciliogenesis or ciliary function. Recently, the presence of a Sec7-related protein in *Tetrahymena thermophila* has been shown