Abstracts

Poster Presentations

P1

Detection of *Cryptosporidium* sp, *Microsporidium* sp. and *Isospora* sp. among the aborigine's school children in Pos Senderut, Kuala Lipis, Pahang, Malaysia

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A total of 180 stool samples from school children of rural area in Pos Senderut, Kuala Lipis, Pahang were examined for the presence of *Cryptosporidium* sp, *Isospora* sp oocysts and *Microsporidium* sp. spores by Dimethy Sulfoxide-Modified Acid-Fast (DMSO) and Gram-Chromotrope staining techniques. The overall prevalence of *Cryptosporidium* sp. and *Microsporidium* sp. infection among the school children was 37.8%. *Cryptosporidium* sp. was detected in 16.7% of the children, 19.6% male and 13.3% female. The prevalence of *Microsporidium* sp was 21.1%, 21.6% male and 20.5% female. *Isospora* was not detected in the samples. The prevalence rate was comparatively higher than previously reported community-based studies elsewhere. There was no significant difference in prevalence rate of *Cryptosporidium* sp. and *Microsporidium* sp. between gender, age and school group level of the children.

P2

Prevalence of intestinal protozoa in an aborigine community in Pahang, Malaysia

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The objective was to estimate the prevalence of intestinal protozoa among the aborigines and to determine the problems causing the infection. The study was carried out in January 2006 in Pos Senderut, Pahang, Malaysia. Samples of feces were collected from children and adults and these were fixed in Polyvinyl Alcohol (PVA) and Trichrome staining was carried out. From the 130 individuals studied, 94 (72.3%) were positive with at least one intestinal protozoa. Nine intestinal protozoa namely *Blastocystis hominis, Giardia lamblia*, *Entamoeba histolytic*a, *Entamoeba coli, Endolimax nana*, *Entamoeba hartmani, Entamoeba polecki*, *Iodamoeba butschlii* and *Chilomastix mesnili* were detected. The most prevalent species was *B. hominis* (52.3%), followed by *G. lamblia* (29.2%), *E. coli* (26.2%), *E. histolytica* (18.5%). The prevalence of the others ranged from 1.5% to 10.8 %. Double infections with *E. histolytica* and *G. lamblia* was 3.8%, *E. histolytica* and *B. hominis* 15.4% and *G. lamblia* and *B. hominis* 17.7%. Triple infection of *E. histolytica*, *G. lamblia* and *B. hominis* was 3.1%. The infection was more prevalent among the age group below 10 years (45.4%) and lowest in the above 60 years (3.8%). The high prevalence is attributable to poor environmental and sanitary management, poor personal hygiene and lack of health knowledge.

P3

Detection of parasitic infection among rural and urban dogs in Peninsular Malaysia

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A study was conducted to determine the intestinal helminthes in dogs from urban and rural communities. Five species of nematodes (*Toxocara* sp., larvae, *Strongyloides stercoralis* larvae, *Ascaris* sp. ova, hookworm ova, *Trichuris* sp. ova, and an indertermined larva) and one species of cestode (*Taenia* sp.) were found from 175 stool samples. Thus 78.3% showed to be positive. Forty-four percent had a mixture of 4 parasites. The prevalence of helminthic infection ranged from 1.1%-43.3%. Prevalence of ascaris was highest among all dogs but hookworm and *Trichuris* gave highest prevalence in urban dogs. Soil samples were also examined to determine the contamination of environment by *Toxocara canis* as a potential source of infection. Urban soil samples showed higher contamination rate. *Toxocara* ova was the most prevalent helminth contaminating the soil. This study showed that human from both urban and rural areas are at risk of acquiring helminth infection from contaminated soil.

P4

Medicinal plants used for the treatment of malaria in Cameroonian folk medicine

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Malaria remains one of the leading public health problems in Cameroon as in other parts of Sub-Saharan Africa. Since some decays, this situation has been aggravated by the increasing spread of drug-resistant *Plasmodium falciparum* strains. Hence, new antimalarial molecules are highly needed. Traditional healers have long used plants to prevent or cure infections conditions. This study attempts to discuss different methods used by traditional doctors in the treatment of malaria in Cameroon, as well as the current status of botanical screening efforts and experimental studies carried out there. Data were collected through interview of traditional healers and from 42 references of diverse research groups; found in the literature up to November 2006. From this study, it was noted that 211 different species have been identified in Cameroon for their use as antimalarial in folk medicine. About a hundred phytochemicals have been isolated from 21 species. Crude extracts and/or essential prepared from 48 other species showed a wide range of activity on *Plasmodium* spp. but bioassay fractionation are needed. Moreover, some 142 plants from 48 families remain uninvestigated for their presumed antimalarial properties. This study shows that Cameroonian flora represents a high potential for new antimalarial drugs. Further studies are needed in ethnobotanical surveys as well as in deep antimalarial and toxicity investigations of identified species.

P5

Detection of insecticides resistance status in *Culex quinquefasciatus* and *Aedes aegypti* from Baan Suan community, Nonthaburi province, Thailand

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Resistance to various insecticides from 4 major groups (organochlorine, organophosphate, carbamate and pyrethroid) was investigated in a field strain of adult female *Culex quinquefasciatus* from Baan Suan community, Nonthaburi province, Thailand by using a standard World Health Organization susceptibility test. The Baan Suan strain was completely resistant to DDT and highly resistant to deltamethrin, permethrin, fenitrothion and propoxur but was found to be highly susceptible to malathion. The study indicated that the Baan Suan strain of *Cx. quinquefasciatus* were resistant to almost all insecticide tested except malathion and this should be an alternative for its control in this area. Moreover, adult *Aedes aegypti*, which is a main dengue vector in Baan Suan community was also tested with deltamethrin, permethrin, fenitrothion and malathion. The results showed that this species was clearly resistant to permethrin and tolerant to deltamethrin, but was 100% susceptible to fenitrothion and malathion. *Ae. aegypti* larvae were also tested with temephos. The cause of insecticide resistance in *Cx. quinquefasciatus* may be due to the continuous use of insecticides for dengue vector control programme in Baan Suan community.

P6

Change of crystal protein formation in *Bacillus thuringiensis* subsp. *israelensis*

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A study on morphogenesis of crystal protein in *Bacillus thuringiensis* subsp. *israelensis* (Pharae strain), was performed to observe the relation between the bacteria at different stages of growth and larvicidal activity. The study comprised construction of growth curve through optical density measurement in CM-02-17 medium, larvicidal activity at time of growth and observation of change on morphology of bacteria cell through electron microscope. This CM-02-17 medium was an appropriate medium because high larvicidal activity against *Aedes aegypti* larvae was noticed as well as it was cheap. Results in the past of observation, cell spent time on lag phase growth about 3 hrs, while exponential phase was in 3-12 hrs after inoculation, after that it went into stationary phase. The larvicidal activity could be observed when the age of culture became 12 hrs. It seemed to be that larvicidal activity increased with increasing age of bacterial culture for certain period of growth. For study on crystal protein that were produced during sporulation, we found that the whole process of spore and crystal protein formation was timed from the end of exponential growth at 12^{th} h of cultivation. This result interrupt with larvicidal property that found cell culture could kill larvae after 12^{th} h of cultivation. In older culture, spore and crystal protein developed until maturity occurred at 27 hrs. After that they are released from bacterial cells above the 39^{th} h of cultivation, in which situation stationary growth results. Furthermore, it is the optimum period for harvest of high larvicidal activity cells.

P7

Evaluation of pyrethrin formulations on dengue/dengue haemorrhagic fever vectors in the laboratory and sublethal effects

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Three formulations of pyrethrin derived from *Tanacetum cinerariaefolium* were evaluated against the dengue/dengue haemorrhagic fever vectors *Aedes aegypti* and *Aedes albopictus* in the laboratory. All the three pyrethrin formulations showed larvicidal and adulticidal activities. The impact of the sublethal doses of pyrethrin formulations on *Aedes* spp larvae resulted in 4-6% of emerging adults alive compared to 90% of *Ae.aegypti* emerging adults and 96% *Ae.albopictus* emerging adults alive in the control. The impact of sublethal doses of the pyrethrin formulations caused very low fecundity on both *Aedes* spp compared to the control($P<0.05$).

P8

Cloning of *Toxoplasma gondii* major surface antigen (SAG1) gene in pTZ57R/T vector and preliminary demonstration of its immunogenicity

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Toxoplasmosis is a cosmopolitan infection which can cause life-threatening disease in the fetus and immunocompromised patient. Thus active research has been carried out to identify effective vaccine candidates against this infection. SAG1 or P30 is an immunodominant antigen of *Toxoplasma gondii* and is considered to be a promising candidate for production of recombinant DNA vaccine against the disease. In the effort to produce such a vaccine, we undertook to clone the *SAG1* gene in a eukaryotic expression vector. *T. gondii* genomic DNA was extracted, and employed in the amplification of the gene. The PCR product was then eluted from the agarose gel, and cloned into pTZ57R/T vector (Fermentas). The recombinant plasmid (pT-SAG1) was prepared from the transformed bacteria (TG1 strain) and sequenced. Sequence analysis showed that the cloned *SAG1* gene has a 100% identity with the sequence reported in the GenBank (AY2177841). The recombinant plasmid was then subcloned into pcDNA3 (Invitrogen) to produce recominant eukaryotic expression plasmid, pcSAG1. Mice injected with the recombinant plasmid elicited a very rapid immunologic response, with high titres of *Toxoplasma* specific IgG, IgM, and IgA antibodies. Therefore, the constructed pcSAG1 recombinant plasmid is potentially useful for further research in designing an effective vaccine against toxoplasmosis.

P9

Anemia: The role of transferrin saturation measurement in patients with chronic renal failure

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Iron deficiency is difficult to diagnose in chronic renal failure patients. The iron status of 70 chronically haemodialysed and 30 non dialysed patients were evaluated by transferrin saturation measurement (TSAT) in this prospective and comparative study. Anaemia was found in 92% and 90% of the dialysis and non dialysis patients' respectively. Iron deficiency anaemia was present in 13/65 in the dialysis and 6/27 in the non dialysis group. Further analysis of dialysis patients with iron deficiency showed functional iron deficiency to be significantly present in 12/13 (92%) of these patients (p=0.035). Considering patients on human recombinant erythropoietin (rHuEPO) therapy, iron deficiency was found in 10/55 dialysis and 3/7 non dialysis patients. All 10 dialysis patients with iron deficiency and on EPO therapy, were found to have functional iron deficiency (p=0.05). Considering dialysis and non dialysis patients as a whole, 59% were diabetic, of whom 58/59 patients were anaemic. Of the 41% non diabetic patients, 35/41 patients were anaemic. The mean haemoglobin of the diabetic patients was significantly lower than non diabetics (p=0.039) when no statistically significant difference (p=0.963) was observed in the mean serum creatinine levels of these two groups of patients. In conclusion this study confirms the value of TSAT measurement to detect functional iron deficiency, a predictable complication in chronic renal failure patients on haemodialysis and on EPO therapy. It also affirms the hypothesis that diabetic patients with renal failure develop anaemia early in renal failure when compared to non diabetics.

P10

Detection of viable *Giardia* cysts by Reverse Transcription-PCR assay

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Giardia, a flagellate protozoan causing diarrhea especially in children worldwide, is transmitted by fecal-oral route from person to person or from animal to person as well as from contaminated food or water intake. Its infective dose is low and the cysts can survive for weeks to months in the environment. Problems associated with detection for parasite is the lack of sensitivity and time-consuming method. The reverse transcription-PCR (RT-PCR) has the potential to solve these problems. We developed a RT-PCR for detecting viable *Giardia* cysts from stool samples by using new primer pairs for giardin beta-subunit. The sensitivity of PCR assay was 5 cysts, whereas RT-PCR assay was 10 viable cysts. Cysts inactivated by freezing or heating were not detected by RT-PCR. This test showed high specificity. The RT-PCR technique is thus recommended as a diagnostic method for detecting viable *Giardia* not only in stool samples but also in environmental water sources.

P11

Effectiveness of Bacillus thuringiensis H-14 against temephos-resistant *Aedes aegypti*

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Aedes aegypti is an important disease bearing mosquito in Thailand. Recently, temephos have been commonly used in the control of mosquito larvae, especially *Ae. aegypti*. Its widespread use has resulted in temephos resistance; posing a serious problem for the control of this species. *Bacillus thuringiensis* H-14 (Bti) can be used for control of *Ae. aegypti* that have become resistant to temephos. Our research was performed to determine the activity of Bti as an alternative larvicide for the control of *Ae. aegypti*. A sample of *Ae. aegypti* from Buriram province, Thailand with a high level of temephos resistance (LC_{90}= 0.03521 ppm., RR_{90}=14.37) and Bora Bora (control) strain were subjected to the study. Insecticide resistance mechanisms were detected. Biochemical tests, polyacrylamide gel electrophoresis (PAGE) and inhibition studies revealed the presence of elevated esterase activity which is associated with temephos resistance. The effectiveness of Bti against the mosquito resistance was observed when mosquito larvae were sequentially exposed to temephos and Bti. The results showed that temephos-resistant larvae showed no resistance to Bti. In conclusion, Bti can be used to eliminate temephos-resistant *Ae. aegypti* mosquitoes.

P12

Evaluation of *Melaleuca cajuputi* essential oil in aerosol spray cans against dengue vectors *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in the laboratory

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The evaluation of the effectiveness of *Melaleuca cajuputi* essential oil in aerosol spray cans as a botanical insecticide against dengue vector was studied. Four different concentration of *M. cajuputi* essential oil were used in the experiment, 1%, 2%, 5% and 10%. A total of 25 adults female mosquitoes were transferred into each 5 net cylindrical cages and were hung in the spraying testing room. Aerosol can were weigh before and after the spraying. Each aerosol was sprayed for 5 and 10 seconds respectively and observation for the knockdown / mortality were made for 1, 5, 10, 15 and 20 minutes respectively. Mosquitoes were then transferred into a paper cup and covered with 'mosquito netting'. After 24 hours the total number of mortality was counted. The results obtained were then compared with the SIRIM STANDARD AEROSOL which contain pralellthrin 0.07% + d phenothrin 0.05% and odourless kerosene 40% LPG 60%. High mortality was observed in both species of mosquitoes among the 5% and 10% of concentrations and those exposed for 5 and 10 seconds. However SIRIM STANDARD AEROSOL exhibited faster and higher mortality in less than 24 hours. There are also no significant ($p<0.05$) difference of susceptibility between *Aedes aegypti* and *Aedes albopictus* whereas the plant extract essential oil showed susceptibility towards *Ae. aegypti* compared to *Ae. albopictus*.

P13

Heterologous expression of the surface antigen SAG2 of *Toxoplasma gondii* in the methylotrophic yeast *Pichia pastoris*

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SAG2 is one of the major surface antigens of the intracellular protozoan parasite *Toxoplasma gondii*. In this study, we used the *Pichia pastoris* yeast expression system to produce a recombinant SAG2 (recSAG2-T) and determined the serological characteristics of this recombinant antigen. We chose the *Pichia* system due to its high efficiency in expressing recombinant genes, and its ability to modify the recombinant proteins. The recombinant antigen was harvested and purified using Mini Prep purification system. The specificity of the protein was tested with toxoplasma positive sera, and then evaluated in western blots. Eighty human serum samples, including 60 from confirmed cases of toxoplasmosis, were tested against resSAG2-T. Results from the western blot assay showed that recSAG2-T reacted with 55 sera from the toxoplasmosis cases but none with the *Toxoplasma*-negative serum samples. The results thus indicate that recSAG2-T was specific for *Toxoplasma* antibody. To further investigate the immunological characteristic of recSAG2-T, this recombinant antigen was injected subcutaneously into mice, and their serum was tested against total protein of *T. gondii*. It was observed that the serum specifically detected the native SAG2 (22 kDa) of *T. gondii*. This shows that the recSAG2-T could evoke the production in mice of antibody which readily recognized the native SAG2.

P14

Preliminary characterization of *Anopheles minimus* CYP6AA3: expression and *in vitro* enzyme assay

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We previously demonstrated that physiological resistance in a laboratory-selected deltamethrin resistant *Anopheles minimus* mosquito was associated with increased detoxification via a P450-mediated mechanism. The cytochrome P450 *CYP6AA3* gene was subsequently isolated from the resistant mosquito. Comparing to the parent susceptible strain, CYP6AA3 mRNA expression level was increased in the resistant strain and during the course of deltamethrin selection. To investigate the role of CYP6AA3 in the deltamethrin metabolism, *in vitro* enzyme assay of CYP6AA3 was preliminarily studied. The full-length CYP6AA3 cDNA was expressed and protein produced via the baculovirus expression system. The expressed CYP6AA3 protein displayed a typical P450 CO-reduced spectrum with the appearance of a 423 nm peak. The amount of expressed CYP6AA3 was estimated from the characteristic spectra and used in the assay reaction. The enzyme activity of CYP6AA3 towards deltamethrin was investigated in the reconstitution assay with *Anopheles* NADPH cytochrome P450 reductase (CPR), and activity was measured by HPLC analysis. Preliminary results of reconstitution enzymatic reactions upon HPLC analysis revealed that CYP6AA3 could play role in deltamethrin degradation.

P15

Biochemical studies of insecticide resistance in *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Thailand

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Twenty-six collections of field collected *Aedes (Stegomyia) aegypti* and eight collections of *Aedes (Stegomyia) albopictus* mosquitoes were obtained from areas across different parts of Thailand. The susceptibility to pyrethroids (deltamethrin, permethrin), organophosphate (fenitrothion) and carbamate (propoxur) insecticides were revealed in these samples. Biochemical analysis was performed on these mosquitoes to determine activities of enzymes including mixed function oxidases (MFO), nonspecific esterases (α- and β-), glutathione-S-transferases (GST), and insensitive acetylcholinesterase (AChE). Biochemical tests were performed on F1generation of *Ae. aegypti* field caught mosquitoes, while in *Ae. albopictus* F2 progenies were used. There was significant enhancement of MFO in pyrethroid resistant *Ae. aegypti* samples. In Nakhon Sawan Province, the north-central part of Thailand, nonspecific esterases conferred fenitrothion resistance in *Ae. aegypti*, while insensitive AChE and/or nonspecific esterases could play role in fenitrothion resistance in Nakhon Ratchasrima (northeastern part of Thailand). There was no consistent association of GST with pyrethroid resistance in *Ae. aegypti*. Low enzyme activities found in *Ae. albopictus* and in *Ae. aegypti* in the south corresponded to their insecticide susceptibility status.

P16

Viability of *Acanthamoeba* spp. cysts after exposure to two hydrogen peroxide systems

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Contact lenses contaminated with *Acanthamoeba* species, a type of free-living parasite are a risk factor for Acanthamoeba keratitis. Thus, contact lens systems play major role for killing amoeba that adheres to the lens and prevents any infection to the eye. The objective of this study is to evaluate the viability of *Acanthamoeba* spp. against two commercially available hydrogen peroxide systems. Three strains of *Acanthamoeba* spp. isolated from clinical specimens and three strains from contact lens paraphernalia were exposed to the two hydrogen peroxide solutions alone and with neutralizing systems. After cultivation of cysts on NNA with overlaid *E. coli*, all strains exposed to the hydrogen peroxide alone could not excyst into trofozoites. After exposure to the disinfecting systems together with their neutralizing kits, some strains were still viable but most of the non-clinical strains were killed. Hydrogen peroxide systems is recommended for disinfecting contact lenses especially when neutralizing system is delayed to allow the disinfecting process.

P17

Contamination of used contact lenses by *Acanthamoeba* spp.

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Acanthamoeba keratitis is a type of corneal inflammation which is associated with contact lens wear. Contact lenses contaminated with *Acanthamoeba* species is known as a predisposing factor for infection to the eye. The objective of this study is to examine used contact lenses of asymptomatic lens users for the presence of *Acanthamoeba* spp. Ninety-nine asymptomatic lens wearers participated in this study. Samples were taken by swabbing contact lenses and cultured onto non-nutrient agar overlaid with *Escherichia coli*. Plates were examined daily for the presence of amoeba. The positive cultures were then confirmed under 'Image Analysis with Video TesT 4.0'. Five of 99 samples (5.1%) were positive for *Acanthamoeba* spp. and all strains identified were from group II (polyphagids) except one from group III (culbertsonids). This finding suggests that *Acanthamoeba* spp. can contaminate the contact lenses of asymptomatic lens wearers thus can be a major risk factor for Acanthamoeba keratitis.

P18

Plasmodium spp. in large fruit bat (*Pteropus vampyrus*)

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Plasmodium is a parasitic protozoa commonly found in red blood cells. The parasite causes malaria in humans and has two hosts in its lifecycle: a mosquito vector and a vertebrate host. Host range among the mammalian order is non uniform. At least 25 species infect primates, a few species are known to infect bats, porcupines, squirrels, birds, reptiles and rodents. Recently, during our study on the blood profile of large fruit bats in a local zoo, female gametocytes and trophozoites of *Plasmodium* spp. were detected in red blood cells of the bats. Bats are infected with many kinds of internal and external parasites. They are known to harbour several protozoans that cause malaria, these include Hepatocystis, Nycteria, Polychromophilus and Plasmodium. Although none of the malarial parasites found in bats cause malaria in humans, there is a crucial need to initiate further investigation to determine the host range and pathogenicity in the bats, as bats can fly more than 100 km per night carrying these parasites.

P19

A survey of Piroplasmosis caused by *Babesia cabali* and *B. equi* in local horses

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Piroplasmosis is endemic in Malaysia and is commonly found in cattle. A survey was conducted to find out the prevalence of *Babesia caballi* and *B.equi* in local horses. The c- ELISA (competitive) antibody test kit was used for this purpose. A total of 450 serum samples of local horses from various equine establishments were tested over a period of 3 months. Results indicate that all the samples were negative for *B.caballi* and 31 samples were positive for *B.equi*. The positive samples were from horses kept in close contact with cattle. None of the race horses from turf clubs or polo clubs tested positive for piroplasmosis. Further work needs to be initiated to control the disease from spreading and causing morbidity in the equine population.

P20

Distribution and parasites isolation of domicilary cockroaches in Kuala Lumpur Malaysia.

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Studies on distribution and parasites of domicilary cockroaches were conducted in 2 urban areas (Pasar Pudu and Pasar Keramat) and 2 suburban areas (Air Panas , Setapak and Salak Selatan) of Kuala Lumpur. Five species of cockroaches were present : *Periplaneta americana*, *Nauphoeta cinerea*, *Blattella germanica*, *Supella longipalpa* and *Symploce pallens*. *P.americana* was the most prominent species found in all studied areas with the numbers caught being 681(52.5%) out of the total of 1298. Parasites were isolated from gastrointestinal tract and body cavity of all cockroaches. The parasites found were *Leidynema appendiculata*, *Hammerschmidtiella diesingi* and *Thelastoma malaysiense*. These species were nematodes from Thelastomatidae family. The other species identified were Spirurid from Spiruridae family, *Moniliformis moniliformis* an acanthocephalan and *Nyctotherus ovalis* a protozoa. The highest prevalence occurred in *Periplaneta americana.*

P21

Field evaluation of controlled-release "strand" formulation of 5% pyriproxyfen against *Aedes aegypti* in household concrete water storage containers in Cambodia

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A second-generation 'strand' controlled-release formulation (5% a.i.w/w) of pyriproxyfen (IGR) developed by Sumitomo Chemical was evaluated. The objective of this field trial was to assess the duration of efficacy against *Aedes aegypti* in concrete water storage containers under field conditions in Cambodia. A total of 100 water storage jars of 300-400 litre capacity in 23 households were used in the intervention arm and 25 water jars in the same village were used as controls. Based on the capacity of the jars when full, 5% strands (135 mg w/w containing 6 mg a.i. per strand) at a treatment rate of 0.039 mg a.i of formulation per litre of water was placed inside water jars. At two-week intervals, pupae were collected from the surface of the water in each treated and control jar using a fine-mesh sweep net and their emergence was monitored in the insectory. The emergence inhibition (%) from the treated jars remained at or above 98% for 24 weeks on 11 of the 12 sampling rounds. After 34 weeks (8 months), the rate of emergence inhibition had declined to 80%. No inhibition was noticed in pupae from the control containers (<5% inhibition). This study demonstrated that the pyriproxyfen 5% slow release formulation, applied at a target dosage of 39 ppb, a.i., effectively inhibited adult emergence of *Ae aegypti* for 6 months in household concrete water storage jars under field conditions. The strand samples collected from the field after 8 months showed wide variations in pyriproxyfen content and weight.

P22

Seasonal abundance of *Aedes albopictus* in selected urban and suburban area in Penang, Malaysia

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Ovitrap surveillance was conducted in selected urban area and suburban area, ie Taman Permai Indah(TPI) and Kampung Pasir Gebu (KPG) in Penang for 14 months. It was found that *Aedes albopictus* was the most abundant *Aedes* species in both study areas, even though a small percentage of *Aedes aegypti* and *Culex quinquefasciatus* was found to breed simultaneously in the same ovitrap. This study indicated that the main dengue vector was *Aedes albopictus*. A strong correlation was found between rainfall and eggs population in both of the study sites (r= 0.982 and r=0.918). The eggs collected were more abundant during low rainfall (dry season) than the high rainfall (wet season).

P23

Change in detoxifying enzyme activities during the different biological stages of *Aedes aegypti* and *Culex quinquefasciatus*

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Insecticide resistance in mosquito is associated with the increasing of three major detoxifying enzyme activities; cytochrome P450-dependent monooxygenase (MFO), glutathione *S*-transferase (GST), and non-specific esterases. In this study, the activities of enzymes were measured from six biological stages of mosquito samples; fourth instar larva, pupa, and 1, 3, 6, and 10-day-old adult female of *Aedes aegypti* and *Culex quinquefasciatus* laboratory colonies. Microplate assay demonstrated variation of enzyme activity profiles in these mosquitoes. The activity of MFO was significantly increased throughout biological stage development in *Ae. aegypti* and *Cx. quinquefasciatus*. In contrast with GST, whose activity was significantly increased in pupa stage of *Ae. aegypti* and in early adult stage (1-day-old) of *Cx. quinquefasciatus*, the activity was gradually decreased in late adult stage (10-day-old). There was no consistent association between non-specific esterases activities, a-esterase and b-esterase, and biological stages were observed in this study. However, the results demonstrated approximately three folds of a and b esterase activities higher in *Cx. quinquefasciatus* than in *Ae. aegypti*. These findings suggest that the biological stages and species dependence of detoxifying enzyme activities could be related to the susceptibility of mosquito to insecticides used in mosquito control.

P 24

Protective effect of palm vitamin E and α-tocopherol against gastric lesions induced water immersion restraint stress in Sprague-Dawley rats

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The present study was designed to compare the effect of palm vitamin E and α-tocopherol supplementations on gastric parameters which are important in maintaining gastric mucosal integrity in rats exposed to water immersion restrain stress (WRS). These include gastric acidity, gastrin level, gastric prostaglandin E_2 (PGE_2) and gastric lesions. Sixty male *Sprague-Dawley* rats (200-250g) were divided into three equal size groups, a control group which received a normal rat diet (RC) and two treatment groups each receiving oral supplementation of either palm vitamin E (PVE) or α-tocopherol (α-TF) at 60 mg/kg body weight. After a treatment period of 28 days, each group was further subdivided into the non-stress and stress groups. The stress groups were exposed to WRS for 3.5 hours once. Blood samples ware taken to measure the gastrin level, after which the rats were killed and the stomach removed to collect gastric juice for analysis. Gastric acidity in the PVE and α-TF stressed groups significantly increased in comparison to the stressed control, but no significant difference was observed when comparing the PVE and the α-TF stressed groups. Exposure to WRS leads to a reduced gastrin level, while the gastrin level in the stressed PVE group and α-TF group were significantly increased in comparison to the stressed control. The gastric PGE_2 content of stressed PVE group and stressed α-TF group were significantly increased in comparison to the stressed control. Gastric lesions of PVE and α-TF stressed groups were significantly reduced in comparison to stressed control.

P25

Larvicidal properties of some Malaysian plants on *Aedes aegypti*

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Aedes aegypti is one of the major vectors in transmitting dengue virus that causes dengue fever(DF) and dengue haemorrhagic fever(DHF). Global resurgence of DF and DHF requires urgency in the control of the vectors. The application of chemicals in vector control programmes pose substantial hazards to the environment in the form of biomagnifications and toxicity to man and other life forms. Extraction of plants were performed with n-hexane and absolute ethanol. The extracts were evaluated for larvicidal properties against late third and fourth stage instars of *Aedes aegypti* var Selangor using the WHO (1981) standard procedure with slight modification. Abate (temephos) as 10 g pure temephos in 1000 g of sand granules was used as positive control. LC 50 of of less than 10 ug/ml was interpreted as with high larvicidal activity. Both hexane and ethanolic extracts of *Mentha arvensis* scored the highest LC_{50} value of 1.24 ug/ml (LC_{90} = 23.36 ug/ml) and LC_{50} of 2.24 ug/ml (LC_{90} = 36.08 ug/ml) respectively. In general the non-polar extracts of these plants have higher larvicidal activities compared to the polar extracts. Plant extracts are potentially valuable alternatives to synthetic insecticides. They are less toxic and environment friendly.

P26

Immunization with recombinant 19 kDa *Plasmodium berghei* merozoite surface protein 1 formulated with alum induces protective immune response in mice

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Despite significant efforts over the past 50 years to control malaria, there are approximately 300-500 million infections and between 1 and 3 million deaths due to malaria. Proteins on the surface of merozoite, such as MSP1, have been considered prime targets for an erythrocyte stage vaccine since they are susceptible to specific humoral immune responses. We investigated the immunogenicity of recombinant rMSP1 (rPbMSP1) that was generated from *Plasmodium berghei*. The rPbMSP1 formulated in alum was found to be immunogenic which induced high levels of specific anti-rPbMSP1 antibody. The IgG2a response predominated over that of IgG1 during the challenge infection in the vaccinated mice. Mice vaccinated with rPbMSP1 in alum mounted significant protective immunity against challenge infection ($P<0.01$). On day 121 after the booster, three out of ten mice immunized with rPbMSP1 in PBS survived parasite infection ($P < 0.05$) and eight out of ten mice vaccinated with r MSP1 in alum did ($P < 0.01$). Hence, obvious protective effects of MSP1 in alum immunization prevented death from *P. berghei* lethal infection in mice ($P < 0.01$). These observations provide an excellent model for clinical assessment of this formulation in human subjects.

P27

Molecular characterisation of the glucose-6-phosphate isomerase gene from *Eimeria tenella*

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Eimeria tenella is an important intracellular protozoan parasite that causes coccidiosis in chickens. Analysis of the structure and function of genes from *E. tenella* would contribute towards our understanding of the complex biology of the parasite. In this study, the gene that codes for glucose-6-phosphate isomerase (GPI), an enzyme that catalyzes the reversible isomerization of D-glucose 6-phosphate to D-fructose 6-phosphate in the glycolysis pathway was isolated and characterised. A pair of gene-specific primers was designed and used to amplify the gene from a cDNA pool synthesized from the total RNA of sporulated oocysts. The PCR product was subsequently purified, cloned and fully sequenced. Analysis of the sequence data indicates that the deduced size of the gene is 1578bp, with an open reading frame that encodes a polypeptide of 525 amino acids in length. Comparison of both the cDNA and corresponding genomic sequences shows that the gene comprises of 15 exons with all exons obeying the "gt..ag" splicing rule of intronic sequences. The *E. tenella* GPI gene sequence exhibits considerable similarities with its counterparts from *Toxoplasma gondii* and organisms of plant origin, suggesting that they are closely related. This is supported by phylogenetic analysis using neighbor joining and maximum parsimony. An understanding of the evolutionary origin of *E. tenella* could pave way for the search of novel and specific chemotherapeutic targets.

P28

Analysis of the chromosome 2 of *Eimeria tenella* reveals a unique repeat organisation

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Eimeria tenella, which belongs to the phylum Apicomplexa, causes avian coccidiosis, an important disease that infects chicken. In order to gain insights into the genome of *E. tenella*, we have sequenced the chromosome 2 of this parasite. Shotgun sequencing of the 1.2 Mb chromosome 2 of *E. tenella* generated 42 contigs, with a total contig length of 822,395 bp and a chromosome coverage of close to 70%. Initial analysis of the *E. tenella* chromosome 2 sequence reveals a segmental repeat organization that is similar to the previously characterised *E. tenella* chromosome 1 sequence - it consists of two repeat rich regions, divided and flanked by repeat poor regions. This repeat organization seems to be peculiar to *E. tenella* as it is not found in other apicomplexan parasites, such as *Plasmodium, Toxoplasma, Theileria* and *Cryptosporidium*. In addition, the telomeric like repeat TTTAGGG/AAATCCC, which is usually found only at the end of the chromosome, was uniquely identified across the *E. tenella* chromosome 2. Further analysis shows that 14.34% of the *E. tenella* chromosome 2 sequence consists of repeats, which is higher compared to most of other apicomplexan parasites.

P29

Oviposition preferences of *Aedes aegypti* to drain water and seasoned tap water

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The oviposition preferences of *Aedes aegypti* to drain water and seasoned tap water were determined in this study. The clear drain water was collected from a stagnant drain in Taman Samudera, Selangor which is a dengue endemic area. Laboratory bred gravid females were given a choice between drain water and seasoned tap water for oviposition. In a no choice test, there was no significant difference in the numbers of eggs, larvae, pupae and adults colonized from drain water and seasoned tap water ($p > 0.05$), indicating that *Ae. aegypti* can oviposit their eggs on a substrate which is readily available. In a choice test, the number of eggs laid by *Ae. aegypti* in drain water (1630.67 ± 204.26) was significantly more than that in seasoned tap water (221.33 ± 53.18) ($p < 0.05$). The number of eggs were 6 folds higher in drain water compared to seasoned tap water. Further more, the oviposition activity index (OAI) was 0.71, indicating that the drain water was more attractive compared to seasoned tap water as an oviposition substrate. This study also shows that pH and BOD value of both drain water and seasoned tap water were not significantly different from each other ($p > 0.05$), indicating that water from the drain did not contain high organic contents i.e. the water was clean and clear. Significant water conductivity ($p < 0.05$) and the presence of bacteria could have contributed to the site selection for egg laying by *Ae. aegypti*.

P30

Ectoparasites of small mammals from urban and forest habitats in Peninsula Malaysia

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Small mammals from urban areas *(Jinjang, Kepong, Setapak, Chow Kit* and *Kampung Keramat)* and forest *(Ulu Gombak Forest Reserve)* were trapped and examined for ectoparasites. Trapped animals comprised of the following : from urban areas- *Rattus rattus diardii(89), Rattus norvegicus (7)* and *Rattus exulans (1)* and from the forest- *Maxomys rajah (12), Sundamys muelleri(10),Rattus bowersi (7), Leopoldamys sabanus (13), Rattus tiomanicus jalorensis (2), Maxomys whiteheadi(3),Lariscus insignis(1), Sundasciurus tenuis (1)* and *Tupaia glis(2).* The following ectoparasites – *Laelaps nuttalli, Laelaps ecidninus, Ornithonyssus bacoti, Notoedres* sp., *Ascoschoengastia indica, Polyplax spinulosa, Hoplopleura pacifica* and *Xenopsylla cheopis* from urban rodents and *Laelaps sanguisugus, Laelaps sculpturata, Laelaps aingworthae, Walchiella oudemansi, Gahrliepia fletcheri, Longolaelaps longulus,* Listrophoridae, *Haemaphysalis* sp, *Ixodes granulatus, Dermacentor* sp. and *Amblyomma* sp. from forest animals were obtained. *Xenopsylla cheopis, Ornithonyssus bacoti* and the ticks (*Haemaphysalis* sp., *Ixodes granulatus, Dermancentor* sp. and *Amblyomma* sp.) are of potential or known medical importance.

P31

Trichomonas vaginalis - distinct differences in the nucleus of trophozoite and 'pseudocyst'

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Trichomonas vaginalis, a flagellated protozoan parasite is commonly found in the genitourinary tract. The sexually transmitted disease is prevalent in approximately more than 200 million people causing a variety of adverse health consequences in both men and women. We have shown previously the existence of 'pseducocyst' of *Trichomonas vaginalis*. The present study through DAPI , acridine orange , Giemsa and Trichrome staining provide evidence of differing staining and fluorescence intensity of the nucleus when trophozites change to the 'pseudocyst' stage. The psudocysts which are described as rounded, compact, non-motile structures without a true cyst wall, and internalized flagella in all isolates exhibit a greater intensity in fluorescence and staining compared to the respective trophozoites. This provides evidence that there is greater accumulation of nuclear material, i.e. probably DNA. The study confirm that the 'pseudocyst' is a definite and distinct life cycle stage which should be included when discussing the life cycle of the parasite. Whether these 'pseudocysts' are the transmissible forms remains in question and warrants further investigation.

P32

Morphological studies of the protozoan parasite, *Trichomonas vaginali –trophozoite to 'pseudocyst' and vice versa*

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Trichomonas vaginalis is a flagellated protozoan parasite. The existence of the cystic stages of the parasite has never been documented and hence the parasite's mode of transmission continues to be in enigma. 'Pseudocyst' when collected from *in vitro* cultures of *T. vaginalis* pooled in large numbers, spun and lysed with distilled water remained as intact rounded structures and were viable for two hours. The sizes of these structures ranged from 7.5µm to 14µm. 'Pseudocysts' were rounded; compact, non-motile structures without a true cyst wall, and possessed internalized flagella. 'Pseudocysts' reverted to the trophozoite stage when cultured in fresh Jones medium. The 'pseudocysts' were not degenerative forms of the trophozoites as previously suggested by others as these structures were found viable using trypan blue. Transformational changes using cytochemical staining revealed that there was a strong correlation between the changes in the intensity of DNA and the structure and morphology of the life cycle stages of the parasite. The study confirms that the 'pseudocyst' stage is a definite life cycle stage with a possible role to enhance the survival potential of the parasite. More correlation study between the intensity of DNA fluorescence and the morphology of the parasite need to be elucidated.

P33

Vero cell line –suitable to propagate *in vitro* growth of microsporidia

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In vitro cell cultures of microsporida can ensure a constant supply of spores for biological and other related laboratory investigation and research. The present study uses 9 different cell lines to assess the susceptibility of cell lines to *in vitro* growth of the spores. Purified fecal samples of microsporidia were inoculated into the following cell cultures to determine their susceptibility to microsporidial infection – Madine-Darby canine kidney (MDCK), African green monkey kidney (Vero), human lung carcinoma (A549), human breast carcinoma (MCF-7), baby hamster kidney (BHK), human pharyngeal carcinoma (Hep2), mouse epithelial myeloma (J558), human ovarian cancer (CAOV3) and pig spleen (PS). The cultures were harvested throughout a 30-day period, where the spores detected in the cells and culture medium were counted. After inoculation with the spores, the cells began to lose their initial confluence on the third day of post-inoculation, and began to swell and cluster together. As cell clumping increased, the integrity of cell membrane began to decrease with dark deposits became visible in some of the cells. Except for the Vero, MCF-7 and A549 lines, the other cell lines failed to thrive despite the addition of fresh cells and died soon after inoculation was carried out. On average, the production of spores in the Vero cells were an average of 25% and 39% better than those in the MCF-7 and A549 cells respectively. These cell cultures may serve to provide a ready source of spores for further studies on their molecular biology and biochemical aspects.

P34

Microsporidia in cancer patients

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Microporidium have been shown to be opportunistic as evidenced by the significant detection in immunocompromised HIV/AIDS population. Cancer patients receiving chemotherapy are known to be immunosuppressed. However, reports of microsporidium in cancer patients have been scanty and sporadic with no study carried out in Malaysia to provide evidence of such a occurrence in this vulnerable population. It is therefore vital to establish the occurrence of this parasite in cancer patients and to assess if it is an opportunistic infection when compared to the prevalence of microsporidia in the normal population. Stool specimens were obtained from 311 cancer patients from three oncology clinics and 173 normal healthy individuals from medical camps (the control group) and screened for microsporidia. Thin smears from each specimen were stained with modified trichrome stain and examined under oil immersion. Microsporidia were detected in 21.9% of the cancer patients and 2.8% of the normal individuals respectively. The spores were visible as ovoid shapes of 1.0-1.5µm, outlined in dark pink against a clear background. A belt-like stripe (the polar filament) girding the equatorial region was visible in some of the spores. The statistical difference between the infection rates in the two groups was significant (p-value $\leq$0.001). It is possible that immunosuppression places cancer patients at increased risk of acquiring such infections. The study underscores the importance of including microsporidia in the screening process for opportunistic parasites in cancer patients as well as other immunosuppressed/compromised individuals as such infections may cause complications.

P35

Elevated level of urinary hyaluronidase in humans infected with intestinal parasites

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Hyaluronidase (Hyase) is an enzyme or glycoprotein broadly expressed in various human tissues and body fluids. Its substrate is hyaluronic acid(HA) which is a glycosaminoglycan that has an important role in the structure of the extracellular matrix. Hyaluronidase may be used by organisms to invade one another and various studies have shown that this enzyme is secreted during the penetration into the host's skin and gut by nematode parasites. Infection by intestinal parasites may cause excretion of urinary hyaluronidase. To date, there have been no studies done on the urinary hyaluronidase of humans infected by intestinal parasites. In this study, the level of hyaluronidase activity in the urine samples from human subjects with intestinal parasitic infection (n=35) and normal healthy individuals (n=95) was determined. Stool examination (for all the subjects) was carried out for the presence of *Giardia lamblia, Blastocystis hominis,* microsporidia, *Dientamoeba fragilis, Ascaris lumbricoides, Trichuris trichiura,* hookworm and *Taenia* sp.. Hyaluronidase level in the subjects with parasitic infection was significantly higher compared to the controls ($P < 0.01$). However, due to small sample size, results were not analyzed according to the types and the burden of intestinal parasitic infection. This preliminary study suggests that the level of urinary hyaluronidase may be used as a marker for the presence of intestinal parasites due to the parasitic invasion activity.

P36

Oxidative stress in parasite infected colorectal cancer patients

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Oxidative stress has been implicated in the etiology of cancer. Various studies have shown host and organ dependent correlation between cancer and parasitic infections. However, little is known about the extent of oxidative stress present in colorectal cancer (CRC) patients with or without parasitic infection are still lacking. In this study, blood sample was used to assess four oxidative indices namely lipid peroxidation (LP), ferric reducing antioxidant power (FRAP), hydrogen peroxide (H_2O_2) and advanced oxidation protein product (AOPP) referring to methods previously established and further modified. Stool examination for the presence of *Giardia lamblia, Blastocystis hominis,* microsporidia, *Dientamoeba fragilis, Ascaris lumbricoides, Trichuris trichiura,* hookworm and *Taenia* sp. was also carried out. Results showed that 36% of the CRC patients had parasitic infection. The levels of AOPP, H_2O_2 and LP in the CRC patients and patients only with parasitic infection were significantly higher ($P<0.001$) compared to the healthy controls. This indicates that oxidative stress is elevated in all the patient groups. FRAP level in the parasite infected patients (without cancer) was almost two fold higher than the healthy controls ($P< 0.001$). AOPP level was higher in CRC patients with parasitic infection and in patients only with parasitic infection (without CRC) compared to the CRC patients without any parasitic infection. The present study provides evidence that the prevalence of parasitic infection in colorectal cancer is high and this also enhances oxidative damage to proteins in the CRC patients.

P37

Mechanisms of resistance to malathion, temephos, permethrin and the variation of esterase activity with the life stages of the mosquito *Aedes aegypti*

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Resistance to organophosphorus insecticides (malathion,temephos) and pyrethroid (permethrin) was studied in insecticide selected strains of *Aedes aegypti* throughout ten generations; results were compared with a susceptible reference strain. The method of larval bioassay and adult bioassay were conducted, revealed that larvae of *Ae.aegypti* malathion selected strain was resistant to malathion at 0.3898 mg/L, temephos 0.0305 mg/L and permethrin 0.2490 mg/L when compared to the susceptible strain. Adult bioassay exhibited *Ae.aegypti* malathion selected strain more resistant at malathion 5.0% (33.7 minutes) compared to permethrin at 0.75% (10.9 minutes). The esterase activity in relation to life stages was studied by using enzyme microassay test. From the results obtained obviously shows that there is a significant difference ($p < 0.05$) in esterase level in malathion, temephos and permethrin selected strains. Larvae of temephos selected strain has the higher level of esterase activity (0.62 – 2.00) α-Na µmol /min/mg protein compared to adult mosquitoes, malathion and permethrin selected strains. Esterase frequencies were very low for malathion and permethrin selected strains (0.08-0.15) α-Na µmol /min/mg protein throughout the life stages. This indicating non-specific esterase is playing an important role in resistance mechanism in larvae of temephos selected strains.

P38

Plasmotomy in *Blastocystis hominis*

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Blastocystis hominis has been regarded as an enigmatic parasite with many aspects of its biology uncertain. Many reproductive processes have been suggested for the organism but to date only binary fission has been proven. Binary fission alone cannot account for the rapid growth rates seen *in vitro* cultures of *B. hominis*. Plasmotomy is one of the modes of reproduction, previously suggested to be seen in *in vitro* cultures. The present study provides photographic evidence for the plasmotomy of *B. hominis* both under light and transmission electron microscopy. The amoeboid form of *B. hominis* was seen to undergo plasmotomy giving rise to two or three daughter cells. Transmission electron micrographs showed these daughter cells to have its own respective surrounding surface coat, mitochondria and vacuoles. Trichrome and acridine orange staining provided further evidence of these reproductive processes seen in the amoeboid forms of *Blastocystis* undergoing plasmotomy. The present study provides evidence of another mode of reproduction apart from the suggested binary fission which probably accounts for the rapid growth rates seen in cultures.

P39

Blastocystis in Ostriches – A possible new species?

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Blastocystis , a widely prevalent protozoan is found in both animals and humans. One of the modes of transmission for *Blastocystis* has been suggested to be zoonotic. There has been only three studies establishing *Blastocystis* in ostriches. The present study analyzed stool samples from 86 ostriches and their respective 8 handlers from a local ostrich farm and a zoo. The prevalence of *Blastocystis* in ostriches and the animal handlers were 80/86 (93.0%) and 1/8(12.5%) respectively using the *in vitro* culture method. The growth profile of *Blastocystis* in culture was distinct and different compared to the other animal and human isolates where parasites peaked on day 2 of culture and thereafter deceased and subsequently to become non-viable. Parasites seen on day 2 cultures showed a mean diameter of 25.5 um with a range between 20-50µm in diameter with vacuolar forms being the predominant stage in cultures. The cysts seen in the feces showed a prominent thick wall surrounding the parasite with large vacuoles and prominent mitochondria. *Blastocystis* isolated from Ostrich animal handlers were morphological and morphometrically identical to *Blastocystis* isolated from Ostriches. Further studies need to be ascertained if zoonotic transmission had taken place. Cysts of *Blastocystis* from Ostriches were morphologically similar to cysts from the handler and the size ranged from 3-4 µm in diameter. The distinct growth profile and exceptionally large vacuolar forms does imply that the parasite isolated from ostriches could be a new species. However, more molecular studies need to be carried out in order to ascertain this fact.

P40

Collection of domiciliary cockroaches from Ringlet, Cameron Highland, Pahang, peninsular Malaysia

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A survey for domiciliary cockroaches was conducted in Ringlet (3600´), Cameron Highland, in Pahang state. Ten beaker traps were set in provision shops and restaurants. Only two traps had cockroaches. *Periplaneta australasiae* (1 female, 2 nymphs) in a provision shop and *Periplaneta brunnea* (2 females, 2 nymphs) in a restaurant were the only cockroach species obtained. No parasites were recovered from specimens dissected. *P. australasiae* and *P. brunnea* are new locality records.

P41

Detection of endosymbionts bacteria from environmental free-living amoebae by transmission electron microscope

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Free-living amoebae (FLA) such as *Acanthamoeba* and *Naegleria* are known to act as reservoirs for a variety of microbial pathogens. In this study, transmission electron microscope (TEM) has been applied to detect the presence and location of endosymbionts bacteria within the FLA. Out of 9 environmental isolates of *Acanthamoeba* (6) and *Naegleria* (3), only 4 *Acanthamoeba* isolates (A1, A2, A6 and N43) were able to be axenized by treatment with 3% HCl and gentamycin (100 µg/ml). These axenic isolates were then maintained in peptone yeast – extract glucose (PYG) medium before being harvested and fixed with 4% glutaraldehyde prior TEM. Results showed the present of endosymbionts both in the trophozoite and cyst stages of *Acanthamoeba*. Most of the endosymbionts bacteria were located either within the vacuoles (2-8 bacteria per vacuole) or freely in the cytoplasm with typical gram-negative cell wall structure of both rods and cocci. Endosymbionts were also detected in the mature cyst in which the extocyst and endocyst wall have been fully developed. The present of dividing bacteria suggested the multiplication of endosymbionts have occurred within those *Acanthamoeba* cells. Attempts to culture the endosymbionts on variety of bacteriological agars including buffered charcoal yeast-extract (BCYE) agar have failed. We conclude that those endosymbionts bacteria detected are host dependent, obligate and need to multiply within the amoebic hosts.

P42

Occurrence of *Blastocystis* in recreational rivers, Selangor

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This study was carried out to detect the presence of intestinal protozoa, *Blastocystis* spp., in two recreational rivers (Sungai Congkak and Sungai Batu) in Selangor, from August 2004 to May 2005. In addition, the determination of its correlation with physical parameter, faecal coliforms count, rainfall and the burden of humans engaged with water-related activities were also carried out. The *in situ* measurement of physical parameters and the collection of water samples were done at 3 sampling stations- upstream, midstream and downstream of both rivers. After cultivation in complete Jone's medium, the water sample from Sungai Congkak and Sungai Batu showed 33% (80/240) and 18% (43/240) of the culture tubes were *Blastocystis* positive respectively. The occurrence of *Blastocystis* showed significant correlation with temperature in Sungai Congkak. While in Sungai Batu, there was significant correlation with dissolved oxygen and turbidity. Analysis by T-test showed that *Blastocystis* had no correlation with rainfall, but significantly correlated with faecal coliform count, and was higher on public holidays compared to week days in both rivers. Among the sampling sites, *Blastocystis* was found higher downstream followed by midstream and lowest at upstream stations. This may due to human activities where settlements and recreational areas of both rivers are located between midstream and downstream stations. Since there was consistent correlation between *Blastocystis* and faecal coliform, therefore, this parameter could be used as indicator for the contamination of river water by *Blastocystis* species. Furthermore, it was known that *Blastocystis* was transmitted by faecal oral route.

P43

Transcriptome analysis of *Lates calcarifer* in response to *Cryptocaryon irritans* infection

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Cryptocaryon irritans an ectoparasitic protozoan, is the aetiology for the marine Ich or White Spot Disease. Being an obligate ectoparasite, *C. irritans* infects virtually all marine teleost including *Lates calcarifer*. In this study, the transcriptome response of *L. calcarifer* towards *C. irritans* infection was investigated using custom *L. calcarifer* cDNA microarray generated from liver and spleen cDNA libraries. The objective of this study was to gain a representative and reliable snapshot of transcriptional response towards infection caused by *C. irritans* using targeted microarray. Parasites infection were carried out on juvenile fish for a period of four days and daily post infection liver's sampling was carried out. Labelled probes were hybridized onto chip from cDNA synthesized from pooled total RNA. Genes induced upon infection were identified and majority of them encode for acute phase proteins (APP). Genes encoded for hepcidin, C-type lectin and serum amyloid A were among those APPs that were highly expressed in infected fish. These results indicate that *C. irritans* induced acute phase response (APR) of *L. calcarifer*. This emphasize on the importance of first line defense shown by the host innate immunity by inducing APR against an ectoparasite infection. The identification of huge number of differentially regulated genes in this study should lead us towards better understanding of response towards infection and immunity in *L. calcarifer.*

P44

Cutaneous toxoplasmosis in an HIV-positive patient

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We report here a case of nodular cutaneous toxoplasmosis in a 49-year-old man confirmed to be HIV positive. He complained of multiple painful nodular lesions on both arms and hands for one year. Serological test for toxoplasmosis was negative. Histopathology examination (HPE) of skin biopsy showed several foci of macrophages with intracellular and extracellular organisms in the underlying dermis. These organisms were crescent shaped, resembling zoites of *Toxoplasma gondii*. The skin biopsy also showed granulomatous inflammation. The histology slide was then examined under electron microscopy and *T. gondii* was identified on the basis of ultrastructural features of the zoites. The organism was confirmed as *T. gondii* in a nested polymerase chain reaction (PCR) which amplified the specific BI gene of the parasite. Cutaneous manifestations of acquired toxoplasmosis are rare and may be attributed to certain strains of *T. gondii*. Skin involvement may be more likely when there is systemic infection or when the patient is immunocompromised. In the case of our patient, the source of infection was probably reactivation of a previous latent infection since he was in an immunocomprised state.

P45

Health effects of exposure to tropical coastal recreational waters

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The health risk posed by poor quality recreational waters generally relate to infections acquired whilst bathing. A questionnaire survey was carried out on recreational users to study health effects of exposure to tropical coastal recreational waters. At the study location, a predetermined number of volunteers were selected. All volunteers underwent visual inspection and pre-test questionnaires were administered. An experimental study design was used whereby the volunteers were divided into 2 groups. The first group was the test group who were required to enter the sea at the selected beach during high tide. After 30 minutes, they exited the water and were visually examined prior to dressing. A post-test questionnaire was administered. The second group, which was the control group, remained at the shoreline between the vegetation line and the water margin. They were allowed to engage in recreational activities for the same of time as the first group. A post-test questionnaire was also administered to this group, along with visual screening. The results, from the follow-up on days 0, 1, 5, and 15, indicated that 20% suffered some untoward health effects in the test group.

P46

Are our tropical coastal beaches clean? – a preliminary report

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In general, coastal beaches are one of the most popular holiday destinations for people the world over. The reason for this is that beaches provide rejuvenating, appealing and aesthetic aspects that attract people to rest and recreate, thereby, enhancing their overall health and well-being. A clean beach is one of the most important characteristics of a waterside resort sought by visitors. Litter on the beach and in marine environments is recognized as undesirable in aesthetic, health and environmental terms, and as a major problem in coastal resource management. A litter survey was carried out at two popular west coast beaches in the Peninsula. A pre-survey assessment was carried out over a period of one week at four beaches to gauge the efficiency and effectiveness of the cleaning process by the municipal authorities and privatized services. A litter survey methodology was developed to determine the type, amount, distribution and source of litter. From our findings of the survey conducted at low tide on two beaches, it was found that biodegradable litter from natural sources made up more than 75% of the total weight of litter collected. The type of litter identified comprised mainly of dried leaves and algae. Man-made litter such as plastics form the other major category collected. Clearly, a two-prong measure is needed to address the issue of generation of man-made refuse: the management of rubbish disposal by municipal authorities and the behavior pattern of recreational users.

P47

Microbiological, entomological and zoological survey of a tropical coastal recreational ecosystem – a preliminary report

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A medico-ecological impact assessment study on raw sea water for parasitic and microbiological parameters, rodents and arthropods was conducted at a popular west coast beach of the Peninsula. The raw sea water was collected from 2 different beach areas during low and high tides. A total of 9 litres of water was collected. From this water sample, 31 copepods of the species *Labidocera jaafari* were collected. This copepod was also collected for the first time at this location. Other organisms found in the sea water were water mites and the skin of razor fly. A total of 200 rat traps were laid for 3 nights at the 2 identified beach areas along the vegetation margin. Twenty-two small mammals were trapped, the majority was rat species. Eight of the rats were found to harbour several species of nematodes and one species of cestode. An ectoparasite survey conducted on the trapped animals showed the presence of mites, lice and chiggers. In the entomological surveys, 6 mosquitoes and 30 other insects were collected by light traps, while in fly traps, 7 flies and 1 wasp were collected. The wasp, *Vespa affinis indosinensis* is medically important. In the bare-leg catch, a total of 59 mosquitoes were collected. Dragging of the sites yielded 24 ticks.

P48

Survey of helminth infection in the wild rats in Sabak Bernam, Selangor, Malaysia

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A survey of helminth infections in wild rats was carried out from May to July 2006. Worms found were preserved in 70% alcohol for nematodes and 4% formaldehyde for trematodes and cestodes before morphologic identification. The ova and larvae were identified using Ethyl Acetate Centrifugal Sedimentation Method. A total of 71 rats (4 species) were caught and 11 species of helminths (8 species of nematodes and 3 species of cestodes) were recovered from these rats. The overall prevalence rate of infected rats was 76.1% and the distributions by species were: *Rattus rattus diardii* (84.0%), *Rattus exulans* (52.9%), *Rattus argentiventer* (50.0%) and *Rattus tiomanicus* (100.0%). The prevalence rates of nematodes were found to be higher than cestodes (77.5% vs 31.0%). The most prevalent helminth infection was Hookworm (56.3%), followed by *Trichostrongylus* sp. (33.8%) and *Ascaris* sp. ova (31.0%). The other species ranged from 4.2% to 28.2%. There were no significant differences comparing between the sex (p=0.290) and the age (p=0.634) of rats infected by the helminth. There were also no significant differences in the length of the body (p=0.111) and tail (p=0.133) between the infected and non infected groups of rats. However, there were significant differences in the length of the hind-foot (p=0.011), length of the ear (p=0.034) and weight (p=0.047) comparing between infected and non-infected rats. Only length of the hind-foot (OR = 1.17, 95% CI : 1.03 – 1.32) and length of the ear (OR = 1.37, 95% CI : 1.01 – 1.85) can be used to be a prediction.

P49

Comparison of chemical drugs on sore and progressive recovery of leishmaniasis in Balb C mice infected with *Leishmania* (L) *major*

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Leishmaniasis (cutaneous and visceral) are reported in Iran. *Leishmania* (L) *major* appeared in Balb/c mice in the form of visceral leishmaniasis with sores. *Leishmania* are intracellular protozoa and multiplies in macrophages. The aim of this research was to study the comparison of chemical drugs on sore and progressive recovery of Leishmaniasis in Balb/c mice infected with *L.L.major*. We choose 4 groups of Balb/c mice (n=6). Theses groups contain: the healthy group as the control and infected group as the case. Parasites (2×10^6) were injected in tail of mice. Sore occurred after 30 days. Two groups were treated daily for 28 days(Glucantime & Amphotricine B). Diameters of sores were measured weekly by caliper. Mice were maintained for 4 months after treatment. The difference between chemical groups was not significant ($P>0.05$). In group treated with Glucanime 70% cure rate occurred and 30% recurred. In group treated with Amphotricine B 65% cured and 35% recurred.

P50

Prevalence of malaria according to the seasons in Varamin city in Iran for one year (2006)

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Malaria exists in 100 countries but is mainly confined to poorer tropical areas of Africa, Asia and Latin America. More than 90% of malaria cases and the great majority of malaria deaths occur in tropical Africa. Malaria is the most important tropical disease, remaining widespread throughout the tropics, but also occurring in many temperate regions. It exacts a heavy toll of illness and death - especially amongst children and pregnant women. It also poses a risk to travellers and immigrants, with imported cases increasing in non-endemic areas. Treatment and control have become more difficult with the spread of drug-resistant strains of parasites and insecticide-resistant strains of mosquito vectors. Health education, better case management, better control tools and concerted action are needed to limit the burden of the disease. In this study we evaluated patients that are doubtful to malaria and refered to health center of Varamin. In these patients, blood smear were examined and the following results were obtained.In spring from 175 doubtful patients, 8 were infected by *Plasmodium* (7 *P. vivax, 1 P. falciparum*). In summer from 175 doubtful patients, 14 were infected by *Plasmodium (13 P. vivax* and 1 mix of *P. vivax* and *P. falciparum*). In autumn and winter the doubtful patients were 30 and 55 respectively, but there wasn't any positive examination for malaria. The results showed that transmission of malaria in Varamin occurs in spring and summer. The information about seasons distribution is important in control programs for malaria.

P51

Cloning and Sequencing of *Leishmania major* Thiol-specific-antioxidant Antigen (TSA) gene

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Leishmaniasis is caused by an intracellular protozoan parasite. *Leishmania major*, is widespread throughout the world. Treatment of this disease is difficult due to toxic and side effects and resistance of available drugs. Development of either new anti-leishmania drugs or a vaccine is an attractive alternative. Genomic of DNA was extracted and used for amplifying of TSA gene as a template. Then PCR product extracted from agarose gel was cloned into PTZ57R/T vector and plasmid containing TSA gene (PTZ57R/T-TSA) was extracted from transformed bacteria (TG1 strain) and sequenced. Enzyme digestion of plasmid extracted from white colonies bacteria (pT-TSA) by restriction enzymes. The plasmids extracted from white colonies bacteria (PT-TSA) were sequenced. Nucleotide sequence analysis of the TSAcloned in pTZ57R/T vector revealed 90% sequence identity and high homology with strain (GenBank Accession No.AFO44679**or** Lmjf15.1080).TSA is the immunodominant antigen of *Leishmania major* promastigote and amastigote being considered as the most promising molecule for a recombinant vaccine or such as DNA vaccine against leishmaniasis. Among the vaccine candidates, TSA(an enzyme homologous to eukaryotic thiol-specific antioxidant proteins) is one of the predominant vaccine candidates. TSA is *L. major* recombinant protein homologue to eukaryotic thiol –specific-antioxidant protein. TSA elicites a Th1 response, stimulated high titers of IgG2a in *L. major* infected BALB/C mice. Compared to selected other antigens. TSA DNA – vaccinated mice showed excellent and stronger protection than the mice vaccinated with the other antigens DNA-vaccinated. TSA production could be a preliminary step for further research in designing diagnostic kit or effective vaccine against leishmaniasis.

P52

Assessing the effectiveness of *Bacillus thuringiensis israelensis* in the control of *Anopheles sundaicus* and *Culex sitiens* breeding in brackish fish ponds

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A fish farm in Pulau Ubin was found breeding with *Anopheles sundaicus* (vector of malaria) and *Culex sitiens* in its fish ponds in June 2005. The Environmental Health Department (EHD) of National Environment Agency conducted a survey of the fish ponds and found that the salinity of the pond water was between 20 - 28 ppt. Such level of salinity is conducive for *Anopheles sundaicus* and *Culex sitiens* mosquitoes breeding. The fish farmer had attempted to use fish oil to control the mosquito breeding in the pond but was ineffective. The Environmental Health Institute (EHI), therefore, initiated a preliminary study using dry formulation of *Bacillus thuringiensis israelensis* granule (VectoBac®WG) to control the *Anopheles* and the *Culex* mosquitoes in the fish ponds. VectoBac® WG was applied to two test ponds (one with about 10,000 fry and another without fish fry but with overgrown algae) at a dosage of 500 g / ha. A back pack mistblower, Stihl 420 SR was employed to disperse the VectoBac® WG into the ponds. The results showed that VectoBac® WG could effectively control the mosquito breeding for 9 days for the pond with 10,000 fish fry, and 5 days for the pond without fish fry but with overgrown algae. No visible impact on the viability of the fish was observed.

P53

Gas chromatographic analyses of chemicals from *Aedes aegypti*

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Pheromone compounds from *Aedes aegypti* mosquito have not been reported in local literature. Chemical compounds from male (250) and female (250) *Aedes aegypti* mosquitoes were extracted with dichloromethane. Dichloromethane was chosen as solvent based on polarity. Extracts were analyzed for chemical compounds using GC-FID and verified with GC/MS analysis. Results obtained were compared. Similarities, prompted further investigations to identify the compounds. Three compounds which could be responsible for communication between the mosquitoes will be discussed.

P54

Effects of *Garcinia atroviridis* on serum lipid profiles in atherosclerotic guinea pig

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The fruit extract of *Garcinia atroviridis*, with solvent methanol, was used to investigate its effect on serum lipid profile in atherosclerotic guinea pig. Twenty four male Dunkin Hartley Guinea pigs were divided to 4 groups. The first group served as control and was given commercial rabbit chow. The second group was forced fed with 1ml *G. atroviridis* (50 mg/ body weight). The third group was given mix feeding (1% cholesterol diet plus *G. atroviridis* (50 mg/body weight). The fourth group was fed with 1% cholesterol diet in food pellet only in order to induce atherosclerosis. After two months of the diet treatment, blood and aorta were taken for biochemical analysis and histological studies. The supplementation of *G. atroviridis* to a high cholesterol diet decreases the levels of cholesterol, LDL in the serum. Histologcal studies showed there is less fat deposition in the aorta of high cholesterol diet animals given *G. atroviridis* compared to the high cholesterol diet animals not given *G. atroviridis*. This study has shown that dietary intake of *G. atroviridis* decreased all lipid composition levels in the serum and it reduced fat deposition in the aorta of high cholesterol diet animals.

P55

Dunnifilaria sp infection in the eye of human: a first case report

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A male filarial worm was recovered from the anterior chamber of the eye of an 80 year old woman in Sabah, Malaysia. She presented with history of pain in the eye associated with blurring of vision. The morphological features are consistent with those of the genus *Dunnifilaria*. However, it is much smaller than the other *Dunnifilaria* reported so far. Growth stunting in abnormal host is likely but a new species could not be ruled out. The adult worm in natural host need to be investigated to determine the species. Transmission dynamics need to be established to assess risk of sustained transmission to human as zoonotic infection

P56

Field trial of rapid test (ICT and Brugia Rapid) for detection of filariasis in Bekasi Regency West Java, Indonesia

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Diagnosis of filariasis relied until recently almost exclusively on detection and identification of microfilariae in night blood specimens. Thus surveys were carried out at night in order detect microfilaria in the population. Recently, immunochromatographic card test (ICT for *bancrofti*), Brugia rapid ™ and ELISA are the suitable techniques and more sensitive test based on daytime blood samples which could provide immediate results, which detect circulating filarial antigens (CFA) in serum or whole blood samples collected during the day. These tests have been shown to be good alternatives to the conventional microscopic technique. ICT has specificity of 100% and a sensitivity of 95%. This study was conducted in Bekasi Regency, one of districts in West Java, which has 65 elephantiasis cases from 2 5 villages. The study was undertaken in March 2005. Eight villages were selected from the 25 potentially endemic villages in Bekasi Regency. For each village, fifty people who live around the elephantiasis case were selected using random selection. The results indicated that out of a total of 400 samples, 2 were found as positive using CFA test while none came out positive using the conventional technique of microscopic examination. The test have shown antigen test to be good alternative to the conventional microscopic test.

P57

Predation of *Aedes* and *Culex* larvae by blood worms Tanypodinae

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Blood worms are known to be free-swimming or crawling predators that burrow in the bottom mud of aquatic habitats. The blood worms that are observed in this experiment are larvae of non-biting midges from the Subfamily Tanypodinae (*Tanypus spp.*). They are found in the stagnant waters of tree holes and man-made containers around rubber plantations in Balik Pulau, Penang. These waters contain mosquito larvae as well. The blood worms were brought back to the laboratory and after a starvation period of 2 days, they were placed individually into containers with 10 mosquito larvae each and monitored for a period of 2 days. The blood worms were observed to prey on the mosquito larvae. The rates of which they attack and consume the mosquito larvae were recorded at predetermined times. Blood worms are ferocious feeders and can consume up to an average of one *Aedes albopictus* larva every 15 minutes. The rates of attack were faster at the 1st and 2nd larval instars compared to the 3rd and 4th instars. Their attack rates were also higher with *Aedes* larvae compared to *Culex* larvae. Therefore, it seems that blood worms have the potential of a biological control agent for container breeding mosquitoes.

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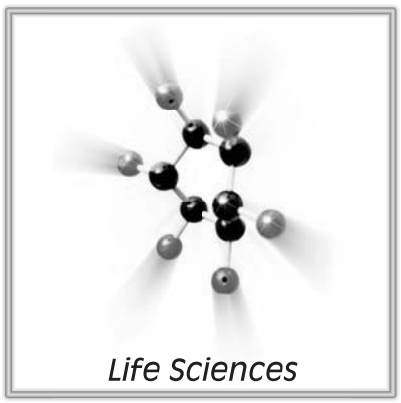
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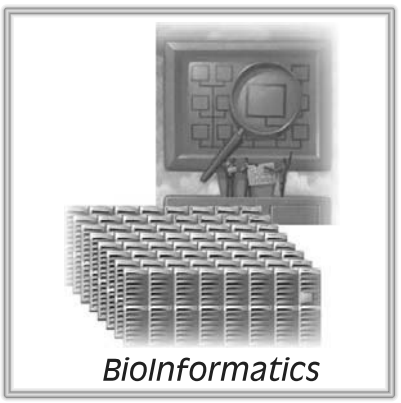
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