THE PARASITOLOGICAL SOCIETY OF SOUTHERN AFRICA

The following are abstracts of papers presented at the Annual Scientific Meeting, 1 - 3 September 1995, Berg-en-Dal, Kruger National Park.

DIE PARASITOLOGIESE VERENIGING VAN SUIDELIKE AFRIKA

Die volgende is uittreksels van referate wat gedurende die Jaarlikse Wetenskaplike Vergadering, 1 - 3 September 1995, Berg-en-Dal, Nasionale Kruger Wildtuin gelewer is.

Strongyloides species from man : differential diagnosis

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Human infection with *Strongyloides stercoralis* is diagnosed almost exclusively by the presence of rhabditiform larvae in the faeces. *Strongyloides fuelleborni* infection is diagnosed by the presence of thin-shelled, hookworm-like, but smaller, eggs (50-58x30-38µm). *S. fuelleborni* eggs invariably contain a longitudinally folded embryo; and are rarely seen at morula stage. Measurement of the embryonated egg will easily distinguish *S. fuelleborni* from the somewhat larger hookworm egg, which may be present in the same faecal specimen.

Free-living adults of *S. fuelleborni* can be recovered from cultures using fresh faeces of any consistency, mixed with super-saturated granulated bone charcoal and left in a warm place. The recovery of free-living adults of *S. stercoralis*, on the other hand, is likely only if the stool is diarrhoeal. Surplus water is pipetted off and replenished every 24h for 3 days. The sediment is allowed to settle and the surplus water pipetted off before 10% formalin is added as a preservative.

In free-living adults, the differences in morphology between *S. fuelleborni* and *S.stercoralis* are most obvious in the females. They include rotation of the vagina to the body axis, the vulval lips and length of isthmus in the oesophagus. Differences between the males are less clear and hinge mainly on minor differences in length of spicules, body and distance between cloaca and tail.

Filariform larvae of *S. stercoralis* and *S. fuelleborni* cannot easily be differentiated.

Is amantadine just another resistance "reversal" agent against *Plasmodium falciparum*?

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The IC_{50} values for amantadine and verapamil are 5,35 µM and 6,4 µM in chloroquine-resistant and 297,82 µM and 21,0 µM in a chloroquine-sensitive strain of *P. falciparum* respectively. Combinations of amantadine or verapamil with chloroquine result in a synergistic drug interaction in the chloroquine-resistant strain, but only amantadine shows synergy in the chloroquine-sensitive strain, verapamil's effect is additive. Both drugs increase uptake of radiolabelled chloroquine in the chloroquine-resistant strain but only amantadine increases chloroquine uptake in the chloroquine-sensitive strain. The increase is shifted, paralleling the difference in drug sensitivity. An amantadine-verapamil combination produces an additive response. The principal mechanisms postulated for "reversal" are (1) a block of the drug efflux pump, P-glycoprotein and (2) alkalinization of the food vacuole. Using rhodamine 123 as a probe for P-glycoprotein activity, we found verapamil in the micromolar range was able to inhibit rhodamine efflux in the chloroquine-resistant strain but had no effect on rhodamine efflux in the chloroquine-sensitive strain. Amantadine did not inhibit rhodamine efflux in either strain at concentrations as high as 1 mM. Acridine orange was used to probe pH changes in the food vacuole. Alkalinization of the vacuole by verapamil was observed in the micromolar concentration range compared with a millimolar range of amantadine. Both the intrinsic antimalarial activity and the mechanism of resistance "reversal" of amantadine appear to be unique.

Evidence of prehistoric parasitism in Late Stone-age coprolite

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A coprolite of late Stone-age man (probable ancestors of modern-day Bushmen) from Kruger Cave near Rustenburg, was examined for helminth eggs. The age of the coprolite was estimated at 7000 - 10 000 years.

The specimen was rehydrated in a 0,5% tri-sodium phosphate solution for 72h. After 24h, acetic-formalin-alcohol (AFA) was added to retard bacterial decomposition and fungal growth. Before preservation in AFA, the sediment was disaggregated by magnetic stirrer and then filtered to remove coarse material. Drops of sediment were stained with dilute Lugol's iodine and examined under a microscope.

Eggs measuring about 48x30µm, with two pale, polar plugs were identified as *Trichuris*. A single, slightly elliptical egg, measuring about 60x50µm and having a thickened wall, was identified as a partially decorticated *Ascaris* egg. A spurious plant parasitic nematode, with a stylet used for penetrating plant tissue, was also found in the specimen.

As far as is known our report is the first from a human coprolite recovered in sub-Saharan Africa.

Dichelyne (Dichelyne) rasheedae Petter, 1974 and *Spirocamallanus olseni* Campana-Rouget and Razarihelissoa, 1965 from fish in Lake St. Lucia, South Africa

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During a survey of fish in Lake St. Lucia, KwaZulu-Natal, South Africa, *Dichelyne (Dichelyne) rasheedae* Petter, 1974 (= *Dichelyne (Dichelyne) fastigatus* of Rasheed, 1968 nec Chandler, 1935) was recovered from the intestine of *Pomadasys commersonnii* and *Rhabdosargus sarba* and *Spirocamallanus olseni* Campana-Rouget and Razarihelissoa, 1965 from *Rhabdosargus sarba*. These are new host and geographical records. Both species were examined by light and scanning electron microscopy which revealed details not previously observed and the results are presented.

Oxidative stress and malaria: A possible protection mechanism against *Plasmodium*

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Oxidative stress may result in severe damage to *Plasmodium* via a mechanism not fully understood. Several suggestions have been made to explain the roles played by reactive oxygen species (ROS) in cell-mediated immunity, malaria pathology and in the inhibition of parasite development. The process of accumulation of redox-active iron degradation products within the parasitized erythrocyte, the role of individual ROS and the role of protective enzymes and scavengers have also not been fully elucidated.

We suggest, in view of past and recent findings (including our own), that the immune response leading to recovery from malaria is associated with effector mechanisms involving ROS. This is particularly significant in abnormal erythrocytes such as G6PD deficient erythrocytes, where sensitivity to ROS could have played a role in evolution. It is also likely that the effector cells, as well as some exogenous factors, induce the production of deleterious hydroxyl radical (OH^-) in a mechanism involving exposure of iron containing structures (ICS) during the development of the parasite. The ICS are known to catalyze the production of the OH^- from O_2^- and H_2O_2. Other free radicals such as NO radical may also be important.

Iron chelators: Mode of action as antimalarials

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Malaria parasites growing inside human erythrocytes differ from mammalian cells in the mode of acquisition of bioavailable iron and in the susceptibility to the anti-proliferative action of iron chelators. We have assessed here three major properties associated with these phenomena: (a) the stage dependent nature of parasite iron mobilization from the host and its integration into parasite proteins; (b) the differential permeability of the plasma membrane to iron chelators and (c) the *in situ* generation of toxic chelator-metal complexes in intracellular milieu of the infected cells.

We have used a combination of synthetic and natural iron chelators with similar iron binding properties but markedly different capacity to permeate across membranes. The profiles of action of these agents on the *in vitro* growth of *Plasmodium falciparum* were assessed in terms of inhibitory concentrations, speed of action, stage dependence and reversibility of effects. These profiles provided the basis for a working model of chelator action on parasitized cells. The model allowed us to predict major improvements in the antimalarial performance of iron chelators when used in appropriate combinations of slow-permeating and fast-permeating substances. The synergistic actions found *in vitro* with various combinations of iron chelators are in accordance with the model and have implications for the design of therapeutic schemes.

Organisms associated with the hindgut of a Spirostreptid millipede

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Millipedes are ecologically important detritivores feeding on decomposing plant material. It was hypothesised that these millipedes, like other detritivores, depend on micro-organisms in their gut to digest the otherwise unutilizable dead plant matter.

Samples from various regions of the gut were collected and routinely processed for light microscopy as well as for scanning and transmission electron microscopy.

Presumably ingested bacteria and fungal filaments occurred scattered throughout the lumen of the fore- and midgut. In the hindgut numerous rod-shaped bacteria and filamentous fungi formed a distinct dense layer which lined the whole epithelial intima. Transmission electron microscopy showed these lining bacteria were associated with cupshaped attachment sites on the chitinous intima. The filaments of Trichomycetes fungi were attached to intimal spines projecting into the lumen. Numerous rhigonematid nematodes were seen scattered among the luminal contents throughout the hindgut with the greatest concentrations occurring just caudal to the pylorus.

The specialized attachment sites in the hindgut of the millipedes may indicate a long mutualistic association with these bacteria and fungi which break down the undigested plant material. Previous reports have considered similar nematodes as being parasites of millipedes.

A scanning electron microscope study of the tropical rat mite *Ornithonyssus bacoti*

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The aim of this study was to investigate the morphological adaptations of *Ornithonyssus bacoti* which enable this small blood-sucking mite to feed successfully on rodents as well as man. Mites of various stages were collected alive and fixed in 70% ethanol. Selected specimens were routinely processed and examined in a Stereoscan electron microscope.

The gnathosoma consisted of a pair pedipalps, a pair of chelicerae with prostrusible chelae and a serrated basal tritosternum. Unique features of the pedipalps included a distinctive "palpal claw" directed medially from the fourth segment and a golfclub-shaped solenidion found medially on the third segment. The "palpal claw" may be used to hold the skin while the chelae are inserted. The pincerlike chelae consist of a fixed spearlike prong and a moveable curved blade to cut through the skin. Each leg ends with a pair of hooked tarsal claws and a pair of comblike tarsal appendages. It is suggested that these interdigitating appendages enable the mite to clasp the hairs of the host firmly during feeding, but allow a quick release for the mite to drop off when the host grooms. This would enable the mite to escape back into the nesting material to feed again another day.

A collaborative project between Department of Medicine, Case Western Reserve University Medical School, Cleveland Ohio and Amoebiasis Research Programme, MRC Durban

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Entamoeba histolytica infects 10% of the world's population. Invasive amoebiasis is one of the leading parasitic causes of morbidity and mortality. It is as yet unknown whether cure of invasive amoebiasis or elimination of asymptomatic intestinal infection is followed by immunity to a recurrent intestinal infection or invasive disease. The aim of the current study is to characterize natural immunity which develops following invasive amoebiasis and asymptomatic intestinal infection with *E.histolytica*.

One hundred patients cured of Amoebic Liver Abscess (ALA) and their 900 close associates will be monitored at 3-monthly intervals for a minimum of three years. The following parameters will be longitudinally monitored: clinical status, occurrence of *E. histolytica* intestinal infection by culture and direct detection of faecal antigen and zymodeme analysis of infecting strain, assays of serum adherence protein antigenaemia, serum IgG antibodies to four major recombinant antigens, salivary IgA to the GIAP and soluble amoebic antigen, CMI studies consisting of lymphocytes - blastogenesis and determination of gamma interferon production in response to mitogens. The progress of this long-term study is discussed.

New concepts for treatment of severe/cerebral malaria

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The pathogenesis of severe/cerebral malaria is not entirely clear but two hypotheses have been proposed: the sequestration hypothesis, cytoadherence and the cytokine hypothesis - TNF, NO.

The probable combination of these two results in cerebral ischaemia, generation of free oxygen radicals and other mediators of inflammation, leads to fast progression of the disease and death of the subject. There is a clear need for a substance which will address both of these problems and at the same time will have a direct effect on parasitic growth. A potential candidate for such a drug is curdlan sulfate (CRDS) which is a sulphated 1 3-β-D Glucan developed and designed as an anti-HIV agent.

It has been shown that CRDS is directly effective in inhibiting the growth of *P. falciparum in vitro* and *P. berghei in vivo*. CRDS has been shown to be synergistic with chloroquine and quinine to down-regulate the immune response by decreasing both TNF and NO. A direct nonspecific effect on cytoadherence may be predicted as has been described previously with other sulphated polysaccharides e.g. heparin. CRDS has minimal systemic toxicity and an anticoagulant effect. Clinical trials are in progress.

Amoebiasis: Resolution of a century old mystery

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When amoebae were first observed in dysenteric stools in the late nineteenth century they were not implicated as the causative organisms. The presence of the same amoebae in bacteriologically sterile liver abscess in 1891 demonstrated their pathogenic potential in the absence of concomitant bacteria. Several names for these organisms were proposed but only in 1903 was the name *Entamoeba histolytica* introduced by Schaudinn. In 1913 Walker and Sellards reported their ground breaking experiments in human volunteers that clearly established the life-cycle and explained the host-parasite relationship. Unfortunately insufficient attention was paid to this work and 70-80 contentious years followed. This was resolved by the pioneering work of Sargeaunt and colleagues who used isoenzyme electrophoresis in the 1980's to prove that a species complex comprising two morphologically identical amoebae was implicated with the disease. These two organisms have been named *E. histolytica* and *E. dispar*. The former is a pathogen and is responsible for invasive amoebiasis, while the latter is a gut commensal. Demonstration of the existence of this species complex has had a major impact on our understanding of amoebiasis and its clinical management.

A comparison of diagnostic serological techniques for the diagnosis of *Entamoeba histolytica*

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The Amoebic Gel Diffusion test (AGDT) is an invaluable economic, reliable and sensitive in-house assay in the laboratory diagnosis of amoebic liver abscess and amoebic dysentery. It is the Gold Standard serological test in the authors' hands and has been in use for more than 25 years. The assay has a sensitivity of 98% and 89% in the diagnosis of Amoebic Liver Abscess and Amoebic Dysentery, respectively. In the assay a 20hr or 40hr precipitin arc is indicative of current tissue invasion or past infection, respectively. The main disadvantage of the test is that it is slow, and, in the quest for a more rapid test which has similar predictive capabilities to the AGDT, a study was undertaken to evaluate 3 commercially available kits. The DTS (Diagnostic & Technical Services cc, Johannesburg, South Africa) Amoebic Gel Diffusion (DTS AGDT) and Entamoeba Elisa (DTS ELISA) test kits available locally, and the imported Indirect Haemagglutination test kit (Cellognost Amoebiasis Combi Pak, Behring, Germany) were compared with the AGDT.

Sera from 105 patients were obtained from the King Edward VIII hospital and these were assayed using all four tests. The AGDT assays were examined at 20hr and 40hr for precipitin arcs, while the ELISA and Cellognost results were evaluated using the manufacturers instructions and formulations. These were then categorised as negative, past infection or current infection and the results and statistical analyses summarised in Table 1 & 2:

Table 1: **Comparative assessment of assays (n = 105)**

	Negative	40hr Positive	20hr Positive
In-House AGDT	32	32	41
DTS AGDT	71	4	30
ELISA	36	25	44
Cellognost	36	12	57

Table 2: **Statistical analysis of results**

	DTS AGDT	DTS ELISA	Cellognost
Sensitivity	45,2%	91,8%	93,2%
Specificity	96,9%	93,75%	96,88%
Pos Predictive Value	97,1%	97,1%	98,6%
Neg Predictive Value	43,7%	83,3%	86,1%

Although all assays had acceptable positive predictive values their negative predictive values varied somewhat, the Cellognost and ELISA tests yielding 2 false negative results compared to 39 of the DTS AGDT. In the in-house AGDT, 32 negative results were recorded while the other 73 samples produced precipitin arcs. The latter corresponds with clinical data.

The ELISA and Cellognost assays provide results within a few hours, while the AGDT can take up to two days. Although the specificity and sensitivity for the ELISA and Cellognost tests were in the nineties, they inaccurately differentiate between current infection (20hr +) and past infection (40hr +). The in-house AGDT consequently remains the most suitable test.

A comparative assessment of the efficacy of a single dose treatment of *Trichuris* infection with albendazole or mebendazole

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Recent estimates indicate that five hundred million people are infected with *Trichuris trichiura* worldwide. The aim of the study was to compare standard single dose treatment of *Trichuris* infection with albendazole and mebendazole in a matched local population of children. The study was conducted in two phases. The first phase was limited to twenty subjects and the second phase to sixty subjects. The study subjects were abandoned or orphaned children aged between 2 and 12 years of age. The selected children were treated with each of the two drugs in a single blinded manner. The results of the pre-treatment and ten day post-treatment quantitative ova counts on the stool specimens were analyzed to assess the efficacy of the two drugs. Percentage reduction was calculated on each patient. In the first phase the average reduction in the albendazole group was 49.2(±33.2%). In the mebendazole group the average reduction was 58.6 (±30,8%). The difference between the two groups was not statistically significant (P=0,49). The results of the second phase of the study are discussed.

Infectious health risks for tourists visiting Southeast Asia and adjacent countries

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An increasing number of South African tourists annually visit countries in Southeast Asia, particularly Thailand. With the "opening up" of the People's Republic of China (P.R. China) and Malaysia to South Africans, the number of tourists is likely to increase still further. An illustrated account is given of the most common health hazards to tourists visiting these countries. Multi-drug resistant *Plasmodium falciparum* malaria is common in the Trat and Tak provinces of Thailand and proper prophylaxis should be taken before entering these areas. The situation is also grave in certain areas in Myanmar, Viet Nam and Cambodia. Other mosquito-borne diseases occurring in these areas include dengue, haemorrhagic fever and Japanese encephalitis.

An account is also given of the danger of acquiring helminth infections in the P.R. China, particularly as a result of the use of "night soil" for the fertilization of vegetables. Traveller's diarrhea caused by *Giardia lamblia* occurs commonly in these countries and there is a risk of infection with *Echinococcus granulosus*, particularly in the northern provinces of the P.R. China and Tibet. Brief mention is made of some pathogens which are less known in the West and may present diagnostic problems in South Africa.

Studies towards the development of a suitable monitoring and control system for *Glossina brevipalpis* and *G. austeni* (Diptera: Glossinidae) in Zululand

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Two tsetse species, *Glossina brevipalpis* and *G. austeni*, are responsible for nagana in cattle in Zululand. A long-term solution to the problem would be to control or eradicate the 2 tsetse species involved.

The attractiveness of certain colours and combinations of synthetic ox-odours (acetone, octenol and phenols) were evaluated by means of electrified grids to develop a target for control purposes. As a monitoring device, the Zanzibar XT sticky trap was tested in various attractive colour combinations.

A mixture of all odour components increased catches of *G. brevipalpis* 2,9 x (significant at P = 0.05) when compared to no odour. None of the odour combinations increased the catches of *G. austeni* significantly.

A combined pthalogen blue and black target was significantly (2-3,5 x) more attractive than an all-black target for both species. The settling response of *G. brevipalpis* was 80-90% higher on the black section of the trap. No apparent colour preference was found in the landing bias of *G. austeni*.

The XT sticky trap was effective in a variety of combinations of black, electric blue (e.blue), light blue (l.blue) and white. The e.blue/black XT increased catches of *G. austeni* significantly (1,5 x) when compared to an e.blue/l.blue control XT. This trap is also effective to catch *G. brevipalpis* and is now used in tsetse distribution surveys.

Monogenean parasites from fishes in St. Lucia, South Africa

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Several species of monogeneans were recovered from marine-type fishes found in St. Lucia lake. Species representing the families Hexabothriidae, Calceostomatidae, Microcotylidae and others were recovered. Species of the genera *Erpocotyle* and *Heteroncocotyle* were recovered from the gills of the bull shark *Charcharhinus leucas*; *Calceostoma* sp. from the snapper kob, *Otolithes ruber; Polylabrus* sp. from the Cape Stumpnose, *Rhabdosargus holupi* and species of *Metamicrocotyle* from the flathead mullet, *Mugil cephalus*. Various other monogeneans were encountered. The morphology, structure of the haptor and mode of attachment of these monogeneans are discussed and presented with the help of light and scanning electron microscopy.

The effects of artesunate on human liver fluke, *Opisthorchis viverreni, in vitro*

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The effects of artesunate and praziquantel on mature and immature *Opisthorchis viverrini* were compared *in vitro*. The mature and immature worms were obtained from infected Golden Syrian hamsters, 3 and 8 weeks post-infection. These worms were placed in a BME medium containing either artesunate or praziquantel and incubated at 37°C in a carbon dioxide incubator. The 2 compounds were used at concentrations of 0, 1, 50, 100, 150 and 200 μg ml^{-1}, and 4 replicates of 3-6 worms each were incubated at each concentration. The worms were removed at 0, 1, 2, 3, 4, 6, 12, 24, 48, 72, 96, 120, 144 and 168 h and changes in their morphology and movement in the BME medium determined.

The motility of mature worms ceased 120, 48, 48 and 24 h after being incubated in artesunate at concentrations of, respectively, 50, 100, 150 and 200 μg ml^{-1}. At none of these concentrations of artesunate did immature worms show any motility after 48 hours. In praziquantel at 1 μg ml^{-1} motility ceased within 1 h. The affected worms showed morphological changes such as vacuolization of the tegument and atrophy of the testes and vitelline glands. Compared to praziquantel, artesunate had to be used at higher concentrations and longer incubation times to cause these changes.

Aspects of *Hyalomma truncatum* nymph embedment in the skin of *Rhabdomys pumilio* under laboratory conditions

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Some immature stages of *Hyalomma truncatum* embed in the skins of their natural rodent hosts while feeding. The objective of this study was to demonstrate and describe this phenomenon as observed in the relationship between *H. truncatum* and *Rhabdomys pumilio* in the laboratory. Twenty *R. pumilio* (trapped at Medunsa, in the Gauteng Province) were infested with *H. truncatum* nymphs (from the tick breeding programme in the Biology Department, Medunsa) and observed daily during the feeding period.

Samples of the embedded nymphs and the surrounding host tissue were used for scanning electron microscopy (S.E.M.), 7 μm thick serial sections stained with Haematoxylin-eosin were used for transmitted light microscopy and 7 μm thick frozen sections of the selected samples were examined histochemically.

S.E.M. studies clearly illustrated the extent of embedment which was obtained in the laboratory. Apart from histochemical changes and extensive polymorphonucleocyte infiltration observed at the feeding lesion, the near absence of a visible immune response in the host's skin lining the pit area indicated its tolerance of the feeding parasites, suggesting a long history of co-evolution in the particular host/parasite relationship.

Recent efforts to determine the distribution of the tsetses *Glossina austeni* and *G. brevipalpis* in Zululand

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In 1953 *Glossina pallidipes*, a savanna tsetse causing widespread losses due to nagana, was eradicated from Zululand. The two remaining tsetse species, *Glossina austeni* and *Glossina brevipalpis*, were then not considered to be involved in the transmission of nagana as they are restricted to dense bush where cattle seldom venture. The serious outbreak of nagana in 1990, however, showed that the situation has changed and that a long-term control approach needs to be developed. Before control can be planned it is essential to determine the distribution of each of the tsetse species.

Experimental work lead to the development of a baited sticky trap suitable for surveying both species. Surveying started in December 1993 and has so far been restricted to game reserves, plantations and commercial farms. Eight 12-day-long surveys have been completed in which 496 traps were set, resulting in 1019 *G. austeni* and 1495 *G. brevipalpis* being trapped on 219 traps.

G. austeni is by far the most widespread species and was found in all the natural areas except the Hluhluwe/Umfolozi Complex and the eastern shores of Lake St. Lucia. At least another year will be needed to survey all suitable habitats in the communal farming areas, as nagana is widespread.

The discovery of cuticular spines of taxonomic importance in the family Lernanthropidae (Copepoda)

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Apart from segmental appendages, copepod parasites have structures that appear to have originated as adaptations to a parasitic mode of life. Being structures additional to cephalic appendages, it is difficult to account for their functional purpose, but they are likely to be useful in attachment to the host. Paired structures located posterior to the antenna are reported from the family Lernanthropidae for the first time. The homology of these structures is uncertain, but similar structures have previously been reported from the Caligidae and Taeniacanthidae.

A number of arguments on the status of these structures being appendages or not had been put forward. Postulations of Wilson (1905, 1911) and Heegaard (1945, 1947) that they were the maxillulae were mainly based on two considerations, viz. the similarity in appearance and the superficial similarity in nerve supply. Counter arguments were put forward by Lang (1946,1948), principally taking ontogenetic sequences into account.

Electron microscopy studies done on species of the genus *Lernanthropus* revealed cuticular outgrowths posterolateral to the antenna. Having a complete set of cephalic appendages, these processes are not derived from limbs, but are probably cuticular spines referred to here as postantennal processes.

The effect of B9303-036 on the susceptibility to chloroquine of *Plasmodium falciparum, in vitro*

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Since the discovery in 1987 that the calcium channel blocker, verapamil, has the ability to reverse chloroquine resistance in *Plasmodium falciparum*, many compounds have been shown to enhance chloroquine susceptibility in resistant strains *in vitro*. However, the mechanism of this reversal activity remains a mystery.

We have examined the effect of B9303-036, a calcium channel blocker, on the chloroquine susceptibility of three chloroquine-sensitive and three chloroquine-resistant southern African reference isolates of *P. falciparum, in vitro* and compared it with that of verapamil. Both B9303-036 and verapamil were shown to have antimalarial activity, although this activity was considerably less than that of chloroquine. The addition of subinhibitory concentrations of B9303-036 resulted in substantial reductions in chloroquine IC_{50} values in the resistant isolates, a smaller reduction in two sensitive isolates and a slight increase in the third sensitive isolate examined. Verapamil had the same effect as B9303-036 in the resistant isolates and in all three sensitive strains verapamil caused the IC_{50} to increase slightly. There was no statistical difference between B9303-036 and verapamil.

The results of this study indicate that B9303-036 is an effective reverser of chloroquine resistance. The use of enhancers of chloroquine activity may well become a reality in the future as the list of antimalarial drugs to which *P. falciparum* is becoming resistant grows.

The use of curdlan sulphate in the treatment of canine babesiosis

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Canine babesiosis is extremely common during the summer months in South Africa. Certain manifestations of disease, such as cerebral babesiosis and so-called "red biliary", have poor prognosis even with intensive treatment. Few novel antibacterials have been investigated since the introduction of diminazene aceturate 30 years ago. Curdlan sulphate, a sulphated glucan polysaccharide, has been shown to inhibit the *in vitro* growth of *Plasmodium falciparum*. It is also known to down-regulate the immune-mediators tumor necrosis factor and nitric oxide, both proposed pathophysiological mediators. A trial comparing the efficacy of curdlan sulphate to the known antibabesial trypan blue was performed.

Four male beagles, all littermates, were infected with the Thomas strain of *Babesia canis*, and were thereafter continually monitored clinically by haematological profiles and peripheral blood smears. The first *B. canis* parasites were observed on blood smears four days after infection, and by the fifth day all four dogs were parasitaemic, but still clinically healthy, with normal appetites. By the sixth day all four dogs appeared clinically sick, with fever, inappetance and mild anaemia. For these reasons, it was decided to treat at midday on the 6th day. Two of the experimental beagles received trypan blue at 10 mg/kg once as an intravenous bolus, while the other two received 5 mg/mg curdlan sulphate, as a 30 minute infusion, every six hours. Before and after infusion the dogs were monitored for temperature, pulse, respiration, habitus and appetite and their PVC and parasitaemia determined. Blood samples were collected at the same time. Full haematological profiles were performed and, because curdlan sulphate is known to inhibit coagulation, partial thromboplastin times were measured.

Results indicate that curdlan sulphate has a lesser antibabesial effect than trypan blue or diminazene aceturate. It appears to attenuate the disease process in several ways, having beneficial effects on appetite, fever and habitus. For these reasons, we believe that the drug shows considerable promise as an adjunct therapy for severe canine babesiosis, and may assist in the treatment of certain forms of canine babesiosis which currently have a poor prognosis.

Evaluation of four techniques in the diagnosis of malaria

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The WHO strategy for malaria control involves "early diagnosis and treatment" which ideally requires a sensitive, quick, simple and cheap diagnostic technique. In South Africa malaria is diagnosed by microscopic examination of Giemsa-stained thick (GTS) and thin (GTnS) blood smears. Three alternative methods were evaluated: the ParaSightTMF (PS) instant ELISA dipstick, the QBCR malaria test and acridine orange staining with Kawamoto's method of thick (KTS) and thin (KTnS) blood smears. In a laboratory study donor blood was infected with cultured parasites. Serial dilutions with known parasitaemia were prepared and sampled.

At Manguzi Hospital, KwaZulu/Natal, 139 blood samples were collected from suspected malaria patients. All samples were examined independently with QBC, PS, GTS, GTnS, KTS and KTnS and by different microscopists. In a field study sensitivity of GTS = 89,1 %, GTnS = 81,8 %, KTS = 94,5 %, KTnS = 81,8 %, QBC = 87,3 % and PS = 96,4 %. Specificity was 100 %, 100 %, 97,7 %, 97,7 %, 100 % and 90,9 % respectively. The 100 % sensitivity cut-off points in the laboratory study were as follows: GTS = 84, GTnS = 1080, KTS = 140, KTnS = 84, QBC = 140 and PS = 30 P/mm3. Total handling time was shortest with PS regardless of whether samples were processed individually or in batches of 10 or 100. Because of its consistently higher sensitivity and speed, the *Para*SightTMF Test is recommended as the diagnostic tool of the future.

The influence of salinity on fish ectoparasite diversity in Lake St. Lucia, South Africa

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Lake St. Lucia, the largest estuarine system in Africa, is a valuable but sensitive ecosystem, requiring sea as well as fresh water to sustain its rich wildlife. The study on parasites of fish from Lake St. Lucia started in 1991, when salinities were close to 0 ppt. In July 1993 the lake reached hypersaline conditions with salinities above 70 ppt.

At low salinity levels (0 to 20 ppt) parasites representing the genera *Ergasilus*, *Dermoergasilus* and *Paeonodes* were collected from mugilid hosts. With salinity in the hypersaline range (40 to 70 ppt) copepods of the genus *Naobranchia* were sampled from *Pomadasys commersonnii* and *Nemesis* and *Croyeria* from *Carcharhinus leucas*. A pennelid of the genus *Penniculus* was also found on the pectoral fin of *P. commersonnii*.

With the salinity in the range of 20 to 40 ppt, the copepods were represented by the families Caligidae, Lernanthropidae, Pandaridae and Lernaeopodidae. The Caligidae included the genera *Sciaenophilus* and *Caligus* of which a number of species were collected from a variety of hosts. A number of species of *Lernanthropus* were also collected from different hosts. The genera *Paralebion* and *Alebion* were attached to *Carcharhinus leucas*. Pandarid fishes were infested with *Alella* and *Neobrachiella*.

The seasonal abundance and prevalence of *Haemoproteus columbae, Leucocytozoon marchouxi* and *Trypanosoma hannae* in the Laughing Dove (*Streptopelia senegalensis*)

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The seasonal abundance and prevalence of haematozoa of the Laughing Doves were studied from June 1992 to the end of October 1994. More than 720 birds were ringed and examined for haematozoa. In addition, more than 340 retrapped birds were examined for changes in their parasitaemia.

Haemoproteus columbae occurred in 42%, and *Leucocytozoon marchouxi* in 17% of the birds caught. No birds were infected with *H. columbae* in December 1992 and the prevalence was low during June 1994. This parasite was more abundant after good rains. The prevalence and abundance of *L. marchouxi* increased after rains. When thin blood smears were examined, 7% of the birds were found to be infected with *Trypanosoma hannae.* This figure increased markedly to over 50% when examining the buffy coat area of centrifuged capillary tubes, which is the more sensitive method. The prevalence was high during summer and low during winter.

The influence of the vectors on the abundance and prevalence as well as the data on the recaptured birds are also discussed.

Canine brain cysticercosis and rabies, a misdiagnosis

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A severe case of cysticercosis of a canine brain is reported. The dog originated from the farm Welgerust in the Vredefort district of the Free State, and became ill 3 weeks prior to its death. Ante mortem symptoms resembled those of rabies and resulted in the dog having to be put down. The brain tested negative for rabies, but multiple cysticerci, *Cysticercus cellulosae,* were found in the brain.

All canids are susceptible to rabies and are also potential hosts for cysticercosis because of their coprophagous habits. Unlike human cysticercosis of the nervous system, which is well documented, the prevalence of cysticerci in suspected rabies infections needs further investigation.

Integrated control of *Haemonchus contortus*: Influence of alternation of sheep and cattle on performance

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It is generally accepted that helminth infection reduces the performance of sheep in terms of growth and wool production, depending on helminth species and the extent of the infection.

A trial was conducted to test whether the alternation of sheep and cattle is useful for controlling particularly *Haemonchus contortus* of sheep and whether different levels of infection have an influence on the performance of the animals concerned. Two replicates of four camps were used. In each replicate, sheep and cattle were alternated in two camps, while the controls consisted of a camp where sheep grazed throughout the year and another where cattle grazed throughout the year. Faecal egg counts (FEC), packed cell volumes (PCV), average daily live mass gains (ADG) and wool production were evaluated. Tracer animals were used to estimate the infectivity of the pastures. The data were tested for interactions between the helminthological parameters and the performance of the animals.

No significant relationships were found that showed dependency of production parameters on the FECs or PCVs, although, with time, high pasture infectivity was followed in turn by a rise in egg counts and lower ADG. The sheep in the alternated camps had a significant improvement in ADG of 16,9 g/day compared to those in the control camps ($P<0,05$). Fleece masses did not show any differences between the camps, but the control sheep had 76% tender fleeces compared to the 28% of the alternated groups. In contrast to these results in the sheep, no differences in performance were found between the treatments in the cattle.

The alternation of sheep and cattle on the same pastures holds promise for both the vet (for worm control) and the farmer (for profitability).