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ABSTRACTS OF PAPERS

A study of malaria in under-fives in Benin City, Nigeria

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Malaria is a common cause of illness and death in children aged 5 years and below in Africa, and there is a paucity of documentation of prognostic indicators for malaria in children of this age group. The study was conducted to compare parasitological, haematological and biochemical indices in children with mild and severe malaria in relation to its outcome. This survey is to report laboratory and clinical prognostic indicators associated with malaria in children in this age group. The subjects were 1565 children seen at the University of Benin Teaching Hospital between January 1998–December 2000. Thick and thin blood smears, stained with Giemsa, were examined and parasite density per microlitre of blood was estimated by multiplying the number of malaria parasites present per 100 high power field by a factor of 500. Haematological and biochemical indices were measured by standard laboratory methods. Of the children studied, 1237 (79.0 %) had malaria, and of these 730 (59 %) and 507 (41 %) had severe and mild illnesses, respectively. Parasite counts at the time of presentation show that patients with severe malaria had significantly higher counts than patients with mild malaria ($P < 0.0001$). The overall mortality was 433 (35 %), of which 372 (30 %) and 61 (5 %) were among severe and mild cases, respectively. Risk factors for fatal outcome malaria were late presentation of sick children at the hospital, age 3 years and below, coma and convulsions. Laboratory indicators for poor prognosis were peripheral schizontaemia, PCV <15 %, blood urea (60 mg/dℓ and blood glucose<30 mg/dℓ. Fever is well recognised by mothers, but features of severe malaria were often ignored. There was increased mortality rate with decreased parental social status. Our results are discussed in relation to control of morbidity and mortality due to malaria in our environment.

Parasites lost and parasites found

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Intestinal parasites cause misery to many millions of less-fortunate people globally, but this tends to be ignored. Diseases caused by parasitic helminths, in particular, are often considered to be of little or no importance. This attitude may exist because worms have been with us since antiquity and are strongly linked to poverty, so that being parasitised by worms is accepted as normal in many communities. More recently, with the devastating HIV pandemic sweeping sub-Saharan Africa and the world, there is startling new evidence suggesting that helminth infection is related to elevated HIV plasmaviral load in dually infected individuals. Also, chronic worm infections down-regulate the immune response, which is needed to prevent infection by the human immuno-deficiency virus, which may also increase the risk of infection by *Mycobacterium tuberculosis.* Recent studies indicate that the efficacy of vaccines against diphtheria, tetanus, cholera and tuberculosis may be compromised by endemic, chronic worm infection. This new evidence has far reaching implications and may thrust helminthology once more into the limelight. This presentation will attempt to contribute to a renewed focus on human helminthoses by means of:

1. An overview of helminths known to infect humans in South Africa
2. A description of some of the pathology that they can cause
3. Discussion on trends in infection rates
4. And consideration of available diagnostic tests for use in humans.

Victory in this silent war may be necessary to achieve effective vaccination against HIV/AIDS and other diseases. It is a difficult war to win because poverty and overcrowding, as well as lack of sanitation and clean water, inevitably expose people to worm infections, which may predispose them to HIV infection. Thus control of human helminthoses may have an impact on prevention of HIV/AIDS and other epidemic diseases in developing countries.

Contribution to the study of the Cestoda, Pseudophyllidea, of *Barbus setivimensis* from Keddara Lake, Algeria

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The present work focuses on the examination of 264 fresh water fishes belonging to the species *Barbus setivimensis* captured from Keddara Lake, 35 km east of Algiers, Algeria. After the dissection of fishes, tapeworms were removed, placed in distilled water, pressed between 2 slides and fixed with 4 % formalin, and preserved in 70 % ethanol. Specimens were stained with acetic carmine and mounted in Canada balsam. Drawings were made with the aid of a *camera lucida* and measurements were taken with an ocular micrometer and are given in millimetres. The study of a minimum of 30 specimens revealed the presence of adult forms and larvae of tapeworms with characters of 2 species of Cestoda, Pseudophyllidea, namely *Bothriocephalus acheilognathi* (adult stage) from the family of Bothriocephalidae and *Ligula intestinalis* (plerocercoid) belonging to the family of Diphyllobothriidae. These parasites were found for the 1st time in Algeria in *Barbus setivimensis.*

The micromorphology of the vulture louse *Falcolipeurus quadripustulatus* (Burmeister 1838)

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Falcolipeurus quadripustulatus belongs to the Philopteridae, a highly specialised ischnoceran group parasitising birds in the Ethiopian region. This family of lice is found on the plumage, usually firmly attached by the mandibles, although they may move sideways across the coverts with amazing rapidity. They are ectoparasites feeding on the epidermal debris and snippets of barbs and barbules. *Falcolipeurus* is parasitic on the Falconiformes and *F. quadripustulatus,* is found on several vulture species. This study is aimed at investigating the micromorphology and to obtain some understanding of how these lice attach to, orientate and feed on their hosts. Lice were collected from disabled Cape griffons kept at the De Wildt Cheetah and Wildlife Centre, Pretoria. The lice were fixed in 70 % ethanol, routinely prepared for SEM and viewed in a Leica Stereoscan 420 scanning electron microscope at 5–10 kV. The studies revealed a long, slender louse that iss dorsoventrally flattened to fit into the grooves between the feather barbs. It has a primitive ischnoceran circumfasciate head, with the oral cavity and mouthparts situated ventrally. The transversely held mandibles are situated on the same level as the antennae and are specialised to grasp and shear off pieces of the feather barbule. Posterior to the mandibles is the labrum, bearing a pair of labial palps terminating in 6 sensory setae. The sexually dimorphic antennae have 5 segments. The 1st segment is enlarged in the male, with a robust, hooked appendage on the posterior margin, while the 3rd segment bears a long curved hook. These hooked processes are used for attachment to the female during copulation. The antenna terminates in a peg organ with 12 sensilla. A plate and a pore organ were visible on the 2 distal segments. Each of the legs terminates in 2 long, curved claws

with the ability to lock over barbules between 3 opposing setae on the pretarsal sclerites. A row of 6 short, robust spines are distinct on each elongated tibia and extend as a row of 8 spines on each elongated femur. This study revealed 2 ventral hooks on the male gonopods while terminal ventral gonopods of the female forms a unique heartshaped plate with 8 distal setae. The SEM greatly enhanced the study of the micromorphology of these specialisations of *F. quadripustulatus.*

Trypanosomosis: proteinases as targets for a vaccine and therapeutic agents

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Trypanosoma congolense, T. vivax and *T. b. brucei,* transmitted by tsetse flies, constitute the major pathogenic trypanosomes infecting ruminants in sub-Saharan Africa. This places 30 % of Africa's cattle and small ruminant population at risk of contracting trypanosomosis. A few trypanocidal drugs are available. The drugs are costly and have considerable unpleasant side-effects. In addition, drug-resistant trypanosomes have been detected over the past 50 years across Africa. This underscores the importance of developing new and improved therapeutic strategies or an effective vaccine. We found that trypanosomal cysteine and serine proteinases are essential for parasite viability and thus focused on these molecules as the targets for the development of therapeutic agents and vaccines. Several chemical compounds, *e.g.* trypanocidal agents and irreversible peptidase inhibitors, inhibit the cysteine proteinase, trypanopain and the serine proteinase oligopeptidase B from *T. b. brucei.* These compounds were also trypanocidal to *in vitro* cultured bloodstream forms of the parasite. Some of these inhibitors protect mice from an otherwise lethal *T. b. brucei* infection. It is difficult to develop a vaccine due to the trypanosomes' ability to escape host immune mechanisms through antigenic variation. We use an alternative immunological approach, aimed at reducing the pathological consequences of trypanosome infection through immunisation against pathogenic factors, *i.e.* proteinases. Trypanotolerant African taurine cattle develop prominent IgG responses to a cysteine proteinase, congopain, upon infection with *T. congolense.* These antibodies, and those induced in susceptible Zebu cattle by immunisation with congopain, inhibited the activity of congopain. Antibody-mediated inhibition of congopain could thus be one of the mechanisms that contribute to trypanotolerance. Therefore, resistance to trypanosomosis may be increased by immunisation with congopain. We are mapping the immunogenic epitopes of congopain to identify protective peptide regions of the protein for inclusion in a trypanosome vaccine. The peptides have potential use for diagnostic assays.

An estimation of the familial relationships among Siphonostomatoida (Copepoda) parasitic on elasmobranchs using molecular data

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The order Siphonostomatoida consists of 19 families parasitic on vertebrates and 21 families parasitic on invertebrates. Of the 19 families parasitic on vertebrates, 12 have been reported from elasmobranchs. Interfamilial relationships among siphonostomes are poorly defined and even the monophyly of the Siphonostomatoida parasitic on vertebrates has been questioned. Benz (1994) used morphological characters to estimate a phylogeny for 18 of the families parasitic on vertebrates, but molecular data has never been used before to study siphonostome systematics. Copepods were collected, from elasmobranchs, at the facilities of the Natal Sharks Board and preserved in 70 % ethanol. DNA extractions were done for 38 specimens from 7 families (Eudactylinidae, Kroyeriidae, Dichelestiidae, Sphyriidae, Pandaridae, Euryphoridae and Caligidae). One nuclear (18S) and 2 partial mitochondrial (*COI* and *16S*) gene regions were amplified and sequenced using an automated DNA sequencer to infer their phylogenetic relationships. Outgroup sequences were obtained from Genbank, and include members of the orders Harpacticoida and Cyclopoida. A sequence for *Lepeophtheirus salmonis* available in Genbank was also included in the analyses. Both maximum parsimony and maximum likelihood criteria were implemented in PAUP as well as a bayesian analysis using MrBayes. The molecular topology differs significantly from the morphological topology for the 7 families.

Survival strategies of *Dipylidium caninum*

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The genus *Dipylidium* Leuckart, 1863, includes the dog tapeworm, *Dipylidium caninum* (Linnaeus, 1758), a cosmopolitan species found in the small intestine of dogs, cats, foxes and humans, mainly children. Intermediate hosts of *D. caninum* are the dog flea (*Ctenocephalides canis*), the cat flea (*C. felis*), the human flea (*Pulex irritans*) and the dog louse (*Trichodectes canis*). Mature *D. caninum* proglottids are excreted with faeces or may leave the anus of the host spontaneously. After expulsion proglottids disintegrate, releasing the egg capsules, each of which contains several fully embryonated eggs. Fleas become infected with *D. caninum* while in their larval stage by feeding on whole proglottids containing numerous egg capsules or on eggs from ruptured proglottids. We hypothesised that the size of the proglottids differs among hosts and that *D. caninum* preferably attaches to a specific area in the intestine. It was further presumed that the number and regularity of the proglottid releases reflect the intensity of the infestation. It was also hypothesised that there is a seasonal periodicity in the release of proglottids that corresponds with seasonality of the intermediate host. Proglottids and scolices from infected dogs and cats were measured and compared. The length of the proglottids from both hosts was more or less equal. Those from dogs were, however, wider compared to proglottids from cats. The number and regularity of expelled proglottids correlated with the level of infection. Attached adult worms were only found in the small intestine of the primary hosts regardless of the intensity of the infection. It was also established that proglottids were expelled in indefinite intervals throughout the photoperiod with no seasonal periodicity.

Resistance of *Boophilus* spp. ticks to acaricides in South Africa

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A survey was conducted to determine the resistance status of field populations of *Boophilus* species in South Africa. In total, 185 field strains were collected and the resistance of ticks against 3 different classes of acaricides, namely an organophosphate (chlorfenvinphos), amidine (amitraz), and synthetic pyrethroid (cypermethrin) were tested. The 'Shaw Larval Immersion Test' was used. The degree of resistance was indicated by a factor of resistance (FOR) and is the number of times the LC_{50} of 1 strain exceeds that of the susceptible strain. The results indicated that 8.6 % of the field populations were resistant to amitraz, 18.3 % to chlorfenvinphos and 34.1 % to cypermethrin. Synthetic pyrethroids were the most common acaricides used by the farmers surveyed. Although amidines were the 2nd most common acaricide used by farmers in this survey the occurrence of resistance was, compared to the other acaricides, the lowest. This suggests a slow rate of resistance development. The use of organophosphorus-containing acaricides by farmers participating in this survey was low. The occurrence of strains resistant to chlorfenvinphos or displaying an emerging resistance was identical at 18.3 % and this probably reflects previous usage patterns and the retention of resistant alleles in the population. This survey confirms that frequent treatments are associated with higher levels of resistance.

Integrated vector management in Africa

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The WHO African Regional Office recognises the need to improve capacity for choosing and implementing vector control methods appropriate to various types of environments in Africa. There is an increasing body of evidence on the efficacy and effectiveness of insecticide-treated materials and indoor residual spraying in specific epidemiological settings. Information on other vector control methods and the conditions in which such methods can be considered cost-effective and sustainable in terms of both entomological and epidemiological impact is less available. There are a number of groups individually engaged in operations research for vector control and environmental management. Some are working in areas of seasonal transmission by *Anopheles arabiensis,* others in areas with perennial transmission by *An. gambiae* s.s., and others working primarily with *An. funestus.* Some projects are rural, others are peri-urban, or on agricultural estates or in industrial areas. Some employ chemical and bacterial larvicides while others investigate the use of environmental management. Given the renewed interest created by the resurgence of malaria and other diseases, it is necessary to optimise vector control by using a rational mix of several tactics with proven effectiveness. This presentation highlights the need to adopt an integrated approach to vector control and management that is cost-effective and sustainable in different ecological settings in Africa.

Reversing sequestration in *Plasmodium falciparum* malaria with antibodies does not work in the treatment of severe malaria

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Malaria is a serious disease infecting some 200 million people annually. Many of the fatalities are children who have cerebral malaria. *Post mortem* studies in these children have revealed the blood vessels supplying the brain and other organs to be packed with malaria-infected erythrocytes cyto-adhering to venous endothelial cells. Cyto-adherence involves the parasite ligands Pfalhesin, sequestrin, adherin and modified band 3 and the host receptors ICAM-1, CD-36, VCAM-1, TRAP, E-selectin and chondroitin sulphate. Adherence can be reproduced in the laboratory. Reversing adherence in humans with anti-malarial antibodies was investigated as an attractive adjunct to the treatment of cerebral malaria. The reversal of cyto-adherence by antibodies both *in vitro* and *in vivo* suggested that a pool of high titre anti-malarial antibodies, shown to contain antibodies to the surface of infected erythrocytes and to reverse adherence *in vitro,* may reverse adherence *in vivo.* The double blind, placebo controlled administration of the antibodies as an adjunct to quinine (the best available antimalarial at the time) had no measurable observed effect on adherence, and did not alter patient recovery. This study evaluates the predicted antibody concentrations required to reverse cyto-adherence. Antibody concentrations were calculated from *in vitro* adherence experiments; the levels of malaria adherence ligands expressed on the surface of infected erythrocytes; the levels of adherence receptors expressed on the surface of endothelial cells and the concentrations of antibodies required to reverse the adherence of cyto-adherent cells in animal models. Results suggest that insufficient antibody was administered in the immunotherapy trial and hence the anticipated reversal of adherence and improved outcome were not seen. The results further suggest that immunotherapy to reverse cyto-adherence is not appropriate as the required concentrations of antibodies are likely to have adverse side-effects.

Functional micromorphology of the slender pigeon louse *Columbicola columbae*

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Infestations with the chewing louse *Columbicola columbae* are common in pigeons worldwide, resulting in feather destruction, weight loss and decreased egg production. This scanning electron microscope (SEM) study aimed at investigating the micromorphology of these lice to obtain some understanding of how they attach to, orientate and feed on their hosts. The lice were collected live from infested pigeons and fixed in 70 % ethanol. After ultrasonic cleaning, they were routinely prepared for SEM, sputter-coated with gold, and viewed in a Leica Stereoscan 420 SEM at 5–10 kV. The head of *C. columbae* is particularly dorsoventrally flattened and lacks the medial groove for holding the barbules seen in many other feather lice. It is further elongated by a flattened anterior plate with specialised sensoria to move between the barbs of the feather. The mouthparts include robust mandibles that grasp a group of barbules to firmly attach the louse to a feather. The terminal surface of each mandible is deeply notched with the inner surface angled to form a sharp cutting surface to shear off pieces of feather for ingestion. A pair of labial palps each bear 6 peglike sensory setae. The thoracic and abdominal spiracles both have slit-shaped luminal openings, which is a unique feature compared to the round spiracular openings of other species of lice. Each leg has 2 tarsal claws. The larger curved claw closes between 4 opposing robust setae of the pretarsal sclerite to firmly grasp the barbules of the feather. The 2nd slightly curved horn-like claw was not observed to close against the pretarsal sclerite but remained open at an angle to the large claw. The mandibles and legs are specialised to prevent the lice from being detached during the rigours of preening and flight. This study confirmed the sexual dimorphism of the 5-segmented antennae. The 1st antennal segment of the male is enlarged while the 3rd segment has a hooklike process for attaching to the female during copulation. Three specialised sensoria were observed on the antennae. These consist of a peg organ with ten uniquely grooved sensillae on the distal tip of the 5th segment while a pore organ with an associated plate organ were observed on the posterolateral surfaces of each of the distal 2 segments. These chemosensory sensoria enable the eyeless lice to orientate on their host, as well as for feeding, mate location and ovipositing.

Caligus parasites collected from marine and estuarine fish in KwaZulu-Natal, South Africa

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Lake St Lucia, on the east coast of South Africa, is the largest estuarine area in southeast Africa and occupies about 80 % of the estuarine area of KwaZulu-Natal. It is 300km^2 in area and has a mean depth of only 1 metre. Lake St Lucia is subject to extreme long-term salinity fluctuations due to its shallow nature and irregular inflow of freshwater, making it a unique estuarine system. Mhlatuzi Estuary is situated about 100 km south of Lake St Lucia and is a much smaller estuary compared to Lake St Lucia. It receives freshwater only from the Mhlatuzi River and its tributaries. Lake St Lucia receives freshwater from 5 different rivers as well as ground-water seepage along the eastern shore. Fish parasitological studies were carried out during 1992, 1993, 1994 and 1997 in Lake St Lucia and in 2001 in the Mhlatuzi Estuary. Many different species of the genus *Caligus* were removed from a wide variety of fish hosts. This genus comprises more than 300 species worldwide, valid or otherwise, of which only 28 species have been recorded from South Africa. Most of the information on this genus from South Africa is limited to very old, more than often incomplete, taxonomic descriptions. These ectoparasites have well-adapted appendages to parasitise a broad host range, contributing to their cosmopolitan distribution. At least 6 different species of *Caligus* parasites were collected during the Lake St Lucia and Mhlatuzi Estuary surveys, *i.e. Caligus acanthopagri, C. confusus, C. epinepheli, C. pageti, C. rotundigenitalis,* as well as an unknown species.

Cross-reactivity between antigens *of Amblyomma cajennense* and *A. hebraeum* (Acari: Ixodidae)

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Laboratory animals on which ticks are fed develop resistance that is reflected by a decline in tick engorgement weight and egg-laying by adults, and reduced egg viability. Serum antibodies from these hosts and their reaction with tick antigens have been detected by different methods, namely precipitation techniques, immunofluorescent techniques, ELISA and Western blots. However, little is known about the effects of antibodies on ticks that engorge on resistant hosts, or which tissues of the tick body are possibly immunogenic. Some researchers, using immunohistochemistry, have detected host antibodies in the gut, salivary glands and hemolymph of ticks engorged on resistant animals. The same technique has helped considerably in determining antigenic sites or antibody targets in other arthropods. In the light of these, we have adopted the immunohistochemistry techniques in order to detect cross-reactivity between sera raised against *Amblyomma cajennense* (Fabricius, 1787) with *Amblyomma hebraeum* (Koch, 1844), and *vice versa*. The results show the existence of shared antigens between the 2 tick species. In general, our results point more to a 1-way cross-reactivity of *A. hebraeum* with *A. cajennense* than actually a reciprocal cross-reactivity, suggesting that *A. hebraeum* is more immunogenic than *A. cajennense*.

Recent advances in the diagnosis of salivarian trypanosomes

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Except for *Trypanosoma cruzi*, all pathogenic trypanosomes belong to the section Salivaria. It is characteristic of the salivarian trypanosomes that, during the course of an infection, a sequence of populations of different antigenic types appears at irregular intervals, which is reflected by the relapsing parasitaemias. In order to develop accurate and sensitive diagnostics of the parasite, target molecules have to be chosen carefully. Therefore, a great deal of effort has gone into discovery of non-variable trypanosome antigens. Ribosomes are cytoplasmic granules (approx. 200 Å) composed of RNA and protein, at which protein synthesis takes place. The P0 protein is an essential component of the eukaryotic ribosomal stalk of the 60s large subunit, and therefore the P0 protein is a non-variable antigen. The ribosomal stalk is directly involved in the interaction of the elongation factors with the ribosome during protein synthesis. Recently, we developed anti-P0 monoclonal antibody clone 4D4 (mAb 4D4), and cloned a cDNA encoding the P0 protein from a *T. congolense* PCF expression library. Epitope mapping of the mAb 4D4 revealed that the P0 has highly antigenic C-terminus polypeptides. Moreover, the mAb 4D4 recognised not only *T. congolense* P0 but also *T. brucei* P0 and *T. evansi* P0 by immunofluorescent antibody test. These results indicate that there is the possibility of development of salivarian trypanosome-specific diagnostics by using the P0 antigen. Therefore attempts were made to produce recombinant P0 antigen (rP0). As a result, the P0 was successfully expressed as a His-tagged recombinant protein in bacterial cell expression system, and purified by using Ni^{2+}-affinity column. Currently we are evaluating an antibody detection ELISA developed by using the rP0.

Clinostomum species infecting the sharptooth catfish, *Clarias gariepinus* in the Okavango Delta, Botswana

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Catfish are hosts and, frequently, intermediate hosts in parasitic life cycles. Flukes that use fish as intermediate hosts generally penetrate the body and encyst in the flesh. The fish are eaten by birds and the larval stage develops into an adult within the bird. During fish parasitological surveys in the Okavango Delta, Botswana, 29 catfish were collected using gill nets. In the field laboratory fish were dissected and the encysted metacercariae removed from the branchial chambers. Some of these metacercarial cysts were excysted and fixed flat using heated 10 % buffered neutral formalin. The remaining cysts were fixed in formalin. In the laboratory, fixed specimens were stained using Mayer's paracarmine for light microscopy and the remaining cysts were critical-point dried, sputter-coated with gold and examined using scanning electron microscopy. Both methods were used for identification purposes. Preliminary studies have revealed that the specimens belong to the family Clinostomatidae and the genus is most likely *Clinostomum* Dollfus, 1950. These parasites are yellow in colour, often giving fish a 'grubby' appearance. In most cases, these worms reach adulthood only if eaten by the proper bird host. Infections by digenetic trematodes have been known to cause mortalities in isolated cases when the number of penetrating larval worms was very high, but a smaller worm burden is not a cause for concern to fish culturists.

Roll back malaria: a South African perspective

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Roll back malaria (RBM) is a new global initiative against malaria. It was established in 1998 in response to the increasing burden of disease and death due to malaria, especially on the African continent. Its objective is to halve the malaria burden in the world by the year 2010. Many partners with diverse strengths and expertise support RBM. In South Africa, the Minister of Health endorsed and launched the South African RBM strategic plan in 2001. This plan is in line with the National Malaria Control Policy and focuses on key strategies and objectives for malaria control in South Africa. The primary objectives are to maintain a case-fatality rate below 0.5 % and to maintain an incidence rate of less than 100 local cases per 100 000 people per year, as measured by annual notifications. The key strategies include: to provide early diagnosis and prompt treatment; to detect epidemics early and contain them; to plan and implement selective and sustainable preventative measures such as vector control and to strengthen capacity in evaluation, basic and applied research. The success to date of these strategies has been documented following the reversal of the upward trend in the number of malaria cases in the 2000/2001 malaria season. A further decrease in the number of malaria cases for the 2001/2002 malaria season has been recorded. This paper provides an update on how the actions outlined in the South African RBM strategy have contributed towards the decrease in the number of malaria cases and how the outlined actions can further contribute towards reaching the targets set by the RBM initiative.

Novel methods in the control of ticks

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Ticks and tick-borne diseases are considered the biggest animal disease problem in Africa. The 4 major genera of ticks are *Amblyomma, Rhipicephalus, Hyalomma* and *Boophilus*. Ticks harm their animal hosts by sucking blood, reducing the growth rate and milk yield, damaging hides and skins, causing tick worry, introducing toxins and by predisposing them to myiasis and dermatophilosis. Ticks also transmit devastating diseases *e.g.* theileriosis, cowdriosis, babesiosis and anaplasmosis to animals. The conventional method of tick control using chemical acaricides is fraught with several problems *e.g.* environmental pollution, chemical residues in meat, milk products and in wool, development of tick resistance and the exorbitant costs. Alternative innovative, environmentally friendly and cost-effective methods of tick control are therefore needed. Some of the available non-chemical strategies that will be discussed include biological control using the entomopathogenic fungi, *Beauveria*

bassiana and *Metarhizium anisopliae,* that can be applied directly on cattle to induce tick mortality, reduce fecundity and egg viability or sprayed on vegetation to infect and kill ticks, especially the immature stages; the parasitic wasp, *Ixodiphagus hookeri,* that can be released on pastures grazed by *Amblyomma*-infested cattle to infect and kill nymphs of *Amblyomma variegatum*; botanicals *e.g.* neem (*Azadirachta indica*) extracts that can be applied topically on the host or mixed with animal feed to repel ticks, deter attachment, feeding, moulting, reduce fecundity and egg viability, and the molasses grass, *Melinis minutiflora,* that can be used as a pasture grass to prevent ticks climbing onto it and attach to cattle. An integrated tick management approach consisting of a combination of several of these strategies will be the most appropriate.

Plasmodium falciparum: In vitro activity of sulphadoxine and dapsone in field isolates from Kenya: point mutations in dihydropteroate synthase may not be the only determinants in sulphadoxine resistance

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Studies of the purified dihydropteroate synthase (DHPS) enzyme *in vitro* and isogenic *Plasmodium falciparum* parasites transfected with different *dhps* alleles have established the importance of point mutations at codons 436, 437, 540, 581 and 613 for sulphadoxine resistance in folate-free medium. However, the primacy of the *dhps* mutations is only observed clearly *in vitro* when the assay excludes para-aminobenzoic acid (pABA) and folate. We determined the relationship between point mutations in the gene that encodes the sulphadoxine target, DHPS and the chemo-sensitivity profile to sulphadoxine and dapsone in folate-free medium among 67 isolates from Kilifi, Kenya. Using nested PCR and enzyme digestion, we assessed the presence of point mutations on the *dhps* domain. The results showed that the *dhps* genotype had a strong influence on the sensitivity to sulphadoxine and dapsone, but that the correlation was far from perfect. Eleven isolates carried a wild type *dhps* allele, but were resistant to sulphadoxine (IC_{50} values >10 μg/mℓ); 4/28 isolates were classed as sensitive to sulphadoxine (IC_{50} values <10 μg/mℓ), but carried a triple mutant (436/437/613) allele of *dhps*. These data show that in low folate medium *in vitro,* the *dhps* genotype alone did not account completely for sulphadoxine or dapsone resistance; other factors such as the utilisation and transport of exogenous folate must also be considered.

The helminth parasites of the most economically important African freshwater fish, *Clarias gariepinus*, and their relation to the fish biology and zoogeography

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The common sharptooth catfish *Clarias gariepinus* is the most widely distributed fish in Africa, found in regions extending from the Nile in the north to as far south as the Orange system. It can reach up to l.5 m in length and up to 59 kg in weight. It is an extremely economically important fish valued in subsistence fisheries, aquaculture and angling. Throughout Africa, it is known by 27 different names. So far, 58 different species of helminth parasites have been reported from this fish representing l9 monogenean, 8 digenean, 7 cestode, 8 nematode and 1 acanthocephalan adult species as well as l5 different larval forms. Apart from the monogeneans, which are skin and gill parasites, most of the other internal helminth parasites of this fish are related to its biology and behavior. This fish is completely omnivorous, preys, scavenges and feeds on available organic food sources, including small fish, birds, frogs, small mammals, reptiles, snails, crabs, shrimps, insects, invertebrates and plant material. Moreover, this species has the ability to live and move outside water as it can breathe atmospheric air and survive desiccation because it is provided with an extra respiratory organ in addition to gills. Through its food, it acquires most of its internal adult species of parasites. The fish is also preyed upon by a wide variety of predators including man, leopards, dogs, crocodiles and birds, especially cormorants, kites, fish eagles and marabou storks. Some of these predators serve as final hosts for the l5 larval forms parasitising the fish host when feeding on it. Fortunately, none of these l5 larval forms infect man, which adds to the economic value and importance of this fish. *Clarias gariepinus* is destined to play an extremely important part in the future of fisheries in Africa to meet the demand for cheap and healthy sources of animal proteins to feed the growing population of Africa. Fish culture has the potential of meeting this increasing demand and already this fish is cultured and farmed in Egypt, South Africa and some other African countries. It is in aquaculture that the importance of and effect of the 19 species of monogenean parasites will be felt and appreciated. In fish culture, parasites particularly the monogeneans, have the chance to multiply and increase in numbers, achieving heavy burdens. Because of the confined water bodies of tanks and ponds, as well as the high density of fish and the close proximity of one fish to another, monogeneans, which have direct life cycles and do not require intermediate hosts, can cause serious problems resulting in the complete loss of the cultured stock. Eight of the 19 monogenean species that infect this fish host belong to the family Gyrodactylidae which is viviparous, producing embryos that already have 2nd and 3rd generation embryos resulting in enormous burdens of parasites in a very short time. Methods of controlling monogeneans are presented and discussed. Clarid fishes are African and Asian in origin and *C. gariepinus* has close zoogeographical affinities to Asian fish belonging to the same genus. The same affinities also apply to the helminth parasites of this fish. Several genera and species of parasites occur only in related hosts in the 2 regions. In fact, one particular digenean species, *Orientocreadium batrachoides* occurs in species of the genus *Clarias* in Africa and Asia. However, some parasites of *C. gariepinus* are entirely endemic to Africa not found anywhere else in the world.

Survival strategies of snail-borne trematode parasites

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It is well known that freshwater snails serve as hosts for trematode parasites. Of these many are medically important, for example *Bulinus africanus* serving as host for *Schistosoma haematobium,* others are of veterinary importance, for example *Lymnaea natalensis* serving as host for *Fasciola hepatica,* while many more snails serve as hosts for various still unknown trematode parasites. The central regions of South Africa are mainly semi-arid and water bodies are mostly temporary in nature. It was thus important to study some of the survival strategies of trematode parasites within this area. Snails were collected by means of metal scoops, cercariae were studied through light and scanning electron microscopy and a spectrum of other invertebrate and vertebrate animals were studied in order to find the next stage (or surviving stage) in its life cycle. Trematode parasites have evolved to survive in, on or outside the host in one of the following ways:

- They live for many years and survive in the final host, for example *Schistosoma* sp. that lives in the body of humans.
- They survive as cysts in the body of the intermediate host, for example the diplostome parasite *Petasiger variospinosus* that lives un-encysted in the pericardial cavity of the 2nd intermediate host *Xenopus laevis laevis.*
- They survive as cysts in the body of the intermediate host, for example echinostome parasites found in the kidneys or heart of *Bulinus tropicus.*
- They survive as cysts on the body of the intermediate host, for example xiphidio parasites encysting on the mantle of *B. tropicus.*
- They survive as cysts on plant material, for example *Calicophoron microbothrium.*

In general parasites make use of animals like *Bulinus tropicus* and

Xenopus laevis which are known to survive periods of draught as well as the extreme climates of hot summers and dry winters in this country.

The occurrence of a novel protozoan parasite in the gut of abalone, *Haliotis midae*: preliminary findings

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The cultivation of *Haliotis midae* is of economic importance in South Africa. Rapid growth of the abalone industry creates the potential for disease outbreaks. In general, there is a lack of knowledge regarding abalone diseases and an attempt was made to address this shortcoming by initiating a shellfish health monitoring programme in 1998. The primary aim of the programme was to reduce the impact of diseases on production. The programme showed the presence of various parasites in abalone, including a novel protozoan infesting the gut. Preliminary examination of the structure of the parasite on light microscopy suggests that it belongs to the phylum Apicomplexa, as no external locomotory organelles have been identified. Scanning and transmission electron microscopy is underway to describe morphological structures for purposes of classification. Data on the occurrence and distribution of the protozoan has been collected over a 5-year period (1998–2002). Preliminary analysis ($n = 3000$) of epidemiological data from 1999 was performed using a chi-square test to compare frequencies of infestation. Various parameters were tested, including the relation between infestation and sex, farm of origin, age and seasonality. The results showed that there was no significant difference in infestation level between males and females. Although some trends indicated higher infestation levels in older animals, this was not statistically significant. Differences in prevalence among farms approached significance. A significant seasonal pattern was not found, but the small data set precluded month by month analysis of data. It is expected that statistically significant differences in parasite distribution over time and among farms will emerge once the entire data set has been included in the analysis.

Excreta and waste disposal practices, the health implication on the poor urban community of Epworth, Zimbabwe

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The International Drinking Water Supply and Sanitation Decade launched by the United Nation's World Health Organisation (WHO) in 1981 emphasised the need to have adequate water supplies as well as good sanitation facilities for all. The concern for human excreta disposal has been growing rapidly, especially in poor urban areas where most of the communities live in over-crowded settlements and are faced with very poor living conditions. The urbanisation process has posed great challenges for governing authorities as regards the provision of sanitation and excreta disposal in many urban areas of developing countries. Most of these areas are already facing critical overload on water resources, improper waste disposal and long lists of service management deficiencies. This study assessed excreta and waste disposal facilities and their impact on sanitation-related diseases in the informal settlement of Epworth on the outskirts of Harare. This was a descriptive cross-sectional community-based study. In total, 308 households were interviewed. Participating households were randomly selected from the 3 communities of Epworth. Secondary medical archival data on diarrhoeal disease prevalence was collected from local clinics and district health offices in the study areas. Only 7 % of households were connected to the sewer system. The study revealed that in Zinyengere Extension, 13 % had no toilet facilities, 48 % had simple pits and 37 % had Blair VIP latrines. In Overspill, 2 % had no toilet facilities, 28 % had simple latrines and 36 % had Blair VIP latrines while in New Gada, 20 % had no toilet facilities, 24 % had simple pits and 23 % had Blair VIP latrines. Although a significant percentage had latrines (83.2 %), over 50 % of the population were not satisfied with the toilet facilities they were using. All the respondents expressed dissatisfaction with their domestic waste disposal practices, with 46.6 % admitting to have indiscriminately dumped waste. According to the community, diarrhoeal diseases were the most prevalent ones (50 %) related to poor sanitation. Health statistics also indicated that diarrhoea was a major problem in this community. It is recommended that households concentrate on improving the provision of toilets, water and waste disposal facilities as a way of improving the health status of the community.

Two exotic species of Monogenea now established in water bodies in South Africa

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This report is part of a major research project aimed at establishing the monogenean fauna of freshwater fish in southern Africa. It deals specifically with the monogenetic parasites of the common carp, *Cyprinus carpio,* which was introduced into this country from northern Eurasia. The parasite fauna of introduced fishes requires investigation to identify the pathogens that have been introduced in the first instance and then to quantify the effects of these parasites on any native host population. Specimens of *C. carpio* were collected from the Hudson Ntsanwisi Dam, Limpopo Province, and the Vaal Dam, Gauteng province, using beach seine and gill nets. Fish were transported live to the laboratory, kept in well-aerated containers and only killed prior to examination. Gills were examined for parasites using a stereomicroscope. Collected parasites were fixed and preserved in 4 % formaldehyde and later mounted in glycerine jelly. *Cyprinus carpio* from the Vaal Dam were found to be infected with *Dactylogyrus extensus* (Muller & Van Cleave, 1932) (100 % prevalence, intensity 12–43 and mean intensity 27) and *Dactylogyrus minitus* Kulwiec, 1927, (prevalence 40 %, intensity 1–3 and mean intensity 2). Specimens from the Hudson Ntsanwisi Dam were found to be infected with *D. extensus* (100 % prevalence, intensity 9–18 and mean intensity 13) and *D. minitus* (100 % prevalence, intensity 15–33 and mean intensity 25). These are parasites of this fish species and have been recorded from it in many countries. Reports show that they are now found on autochthonous fish species in countries where the fish was introduced. In Egypt, for instance, *D. minitus* was recorded from *Labeo niloticus* and *D. extensus* from *Schilbe intermedius.* These 2 parasites are now well established in the 2 dams in South Africa.

Prevalence of porcine cysticercosis and hydatidosis in slaughtered animals in southwestern Zimbabwe: a retrospective study

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The aim of this study was to investigate the prevalence of porcine cysticercosis and hydatidosis in pigs, sheep and goats slaughtered at the Bulawayo Colcom abattoir situated in southwestern Zimbabwe. Retrospective data was collected from the abattoir for the period 1994 to 2001. Source of the slaughter animals was taken into account during compilation of cysticercosis data. The mean prevalence rates of porcine cysticercosis for the different years were as follows; 1994, 0.18 %; 1995, 0.16 %; 1996, 0.16 %; 1997, 0.14 %; 1999, 0.54 %; 2000, 0.49 %; and 2001-0.48 %, while the overall mean prevalence rate was 0.34 %. There were no significant differences in mean prevalence of cysticercosis according to source of pigs, year, month, and province of origin. The prevalence rates according to source of pigs were as follows: commercial, 0.18 %; small-scale-, 0.66 %; and rural, 0.14 %. The prevalence rates of hydatidosis in sheep and goats for the different years were 1994-24.4 %, 1995-12.5 % and 1996-35.95 %, while the overall mean prevalence rate was 16.7 %. The prevalence rates of hydatidosis in pigs were 1994-0.4 %, 1995-0.35 %, 1996-0.23 %, 1997-0.35 %, 1999-0.51 %, 2000-0.58 % and 2001-1.41 %. The mean prevalence rate of porcine hydatidosis was 0.46 %.

Concurrent infections of both liver and lung were less common than infections of liver or lung alone. There were no significant differences in the occurrence of hydatid cysts in the liver and lung of pigs, unlike in sheep and goats where the prevalence was significantly higher in lung than in liver ($P < 0.05$).

Anthelmintic treatment: the use of unregistered products and their role in underdosing

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The use of anthelmintic products in the general care and management on horse breeding farms is commonly practiced. Regular evaluation of these products ensures that effective drugs are used. The aim of this study was to evaluate the efficacy of doramectin, pyrantel pamoate, ivermectin and moxidectin on a thoroughbred horse-breeding farm in the Western Cape Province. The study involved 30 yearlings and 40 weanlings of mixed sex in 2001 and 2002, respectively. In each of the years the horses were divided into 3 and 4 groups of equal size. In 2001, moxidectin was 1 of 3 drugs administered orally and at a dose rate of 0.4 mg/kg. In 2002 pyrantel pamoate, ivermectin and moxidectin were orally administered at 19, 0.2 and 0.2 mg/kg. Doramectin was administered by intramuscular injection at a dose of 0.2 mg/kg. The faecal egg count reduction test was used to determine the treatment efficacies in both years. Each animal was used as its own control and the arithmetic mean faecal egg count and lower 95 % confidence limit was calculated for each of the groups. A 100 % reduction in the faecal egg counts and a 100 % lower 95 % confidence limit was recorded for moxidectin at 0.4 mg/kg. A 99 and 96 % reduction was recorded for pyrantel pamoate and ivermectin, respectively. Doramectin and moxidectin resulted in no reduction following treatment. Only pyrantel pamoate recorded lower 95 % confidence limits above 90 % of the 4 drugs tested in 2002.

Disease-causing agents in free-ranging poultry from the Qwa-Qwa district of the Free State province, South Africa

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Disease-causing agents of free-ranging poultry in the Qwa-Qwa district of the Free State province were investigated over a period of 6 months. Blood was collected from the wing veins of 177 poultry in 17 villages for serodiagnosis, preparation of blood smears and determination of packed cell volume (PCV). Coccidia and helminths were identified from fresh, pooled faecal samples collected from poultry houses using MacMaster and Visser sieve techniques. Fifty-two percent of chickens tested for infectious bronchitis were seropositive. The range of PCV values for chickens were 15–39 %, for ducks 13–36 % and for geese 13–29 %. Helminths eggs isolated were *Ascaridia, Capillaria* and *Trichostrongylus* species. *Eimeria* species were also isolated. No haemoparasites were identified. Ectoparasites included the red fowl mite, *Dermanyssus gallinae,* sticktight flea, *Echidnophaga gallinacea,* and the louse *Menopon gallinae.* To ensure a thriving and sustainable free-ranging system in Qwa-Qwa, the small-scale farmers will require assistance in the control and prevention of disease-causing agents through vaccination and administration of drugs to infected poultry.

Preliminary studies on the prevalence and distribution of urinary schistosomiasis in Ondo State, Nigeria

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A survey of the prevalence of schistosomiasis, in all 18 Local Government Areas in Ondo State, was conducted from March to May 2001. Sixty urine samples were collected from each of the 4 primary schools randomly selected in each Local Government. These samples were later examined under the microscope for schistosome eggs. Questionnaires were also distributed to children in classes 2–6. Snail sampling was done using scoops at possible contact sites. The survey covered 70 schools where 4265 urine samples were collected and analysed. The descending order of prevalence is: Ileoluji-Okeigbo (68.6 %) > Akure North (54.2 %) > Akure South (54.1 %) > Odigbo (48.6 %) > Ifedore (47.3 %) > Idanre (42.9 %) > Eseodo (42.2 %) > Ondo East (23.3 %) > Irele (21.9 %) > Akoko South-West (21.3 %)> Owo (19.6 %) >Akoko South-East (3.8 %) >Ilaje (3.38 %). More males than females were generally infected with *S. haematobium. S. mansoni* eggs were encountered in the urine in some areas during the survey. Subjects found positive were treated with praziquantel. Information from the questionnaire was grouped according to age, sex, whether they pass blood in their urine or not, their sources of water and water contact activity. The questionnaire showed that Ileoluji-Okeigbo had the highest cases (82.6 %) of blood in urine (haematuria) and Akoko South East had the lowest (2.5 %). There was a varying degree of dependence on each of their sources of water, which included rainwater, wells, streams, taps/boreholes and ponds. Bathing activity, one of the contributory factors in transmission, was prominent in Okitipupa, Ondo West, Akoko South West, Ileoluji-okeigbo, Akure South, Ifedore and Ondo East. *Bulinus globosus* was the predominant intermediate snail host while *Biomphalaria pfeifferi* was recovered from 3 water-contact sites in the state. The high prevalence of schistosomiasis in this study requires an urgent solution.

Determination of cut-off p(LDH) optical density levels for field evaluation of malaria in Kenya

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Healthy subjects (controls) from a malaria non-endemic area and subjects, both field and clinical, in a malaria endemic area of Kenya were recruited and their plasma and red blood cells measured using the modified p(LDH) procedure. Plasma and red blood cells p(LDH) for the controls were significantly lower than those of subjects from field and clinical studies. The clinical studies, using thick and thin blood films, as well as *t*-statistic test (for thick and thin films), to determine cut-off optical density for red blood cells, gave the optimum sensitivity values as 62.9, 77, 60 and 78.4 %, respectively and specificity's of 69, 80, 39.4 and 28.6 %, respectively. For plasma, the sensitivity values were 61.7, 75.6, 61.5 and 75.2 %, respectively and specificity's of 71.4, 30.7, 46.2 and 26.1 %, respectively. The use of the *t*-statistic to determine cut-off consistently gave lower specificity (46.2 *vs* 71.4 % for plasma, 60 *vs* 62.9 % for RBC) in clinical samples. In field studies, the methods were largely agreeable in specificity (45 *vs* 44 % for plasma, 64.3 *vs* 42.5 for RBC) and sensitivity (73.7 *vs* 89 for plasma, 60.3 *vs* 57.9 for RBC). This is the only *in vivo* study trying to correlate red blood cell and plasma p(LDH) with microscopy using subjects of 3 different categories: healthy non-infected individuals outside malaria endemic region (control group 1, $n = 107$), non-parasitaemic healthy individuals outside malaria endemic regions (field A = 0, B = 0), parasitaemic non-symptomatic individuals in endemic region (field A = 1, B = 1), (both field study group 2) and non-parasitaemic symptomatic individuals in endemic region clinical A = 1, B = 1), (both clinical study group 3). The results of this study indicate that *t*-statistics can be used to evaluate malaria p(LDH) cut-off in field studies.

Notes on the life-cycle of the digenean (Trematoda: Hemiuridae) occurring in *Haliotis spadicea* (Donovan, 1808)

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Larval stages of digenetic trematodes are often associated with

limpets. The rediae of *Cercariae patellae* (Lebour, 1911) in *Patella vulgata* Linnaeus, 1758, are known to invade the ovotestis and either consumed the gonad or produced a reduction of the germinal epithelium. Recently, it was found that *C. patellae* is a developmental stage of a digenean found in the intestine of the oystercatcher, *Haematopus ostralegus,* and should be referred to as *Echinostephilla patellae* (Lebour, 1911). *Cercariae patellae* has been recorded from the granite limpet, *Cymbula granatina* (Linnaeus, 1758), in South Africa. Specimens of the bearded limpet, *Scutellastra barbara* (Linnaeus, 1758), were examined at the De Hoop Nature Reserve on the south coast and were also infected with *C. patellae.* The possibility exists that the cercariae reaches maturity in the host *Haematopus moquini* (African black oystercatcher). These birds have a large protected breeding community at the De Hoop Nature Reserve. Venus ears, *Haliotis spadicea,* (Donovan, 1808) were also collected and dissected. Cercarial, redial, metacercarial and adult stages of a digenean trematode were found in the digestive glands as well as on the gill filaments. Gills were fixed in 10 % buffered, neutral formalin for SEM. In the laboratory the specimens were prepared for SEM using standard techniques and micrographs were taken to measure body dimensions. Data collected from 1999–2002 shows an extremely high prevalence of 100 %. These digeneans belong to the family Hemiuridae (Looss, 1899). Hemiurid larval types have previously been found in the false limpet Siphonaria and Burnupena from South African shores. In *H. spadicea,* however, some adult stages were also found, suggesting that these hemiurids have a single-host life-cycle.

An overview of the reproductive process, conjugation, in *Mantoscyphidia branchi* (Van As, Basson and Van As, 1998)

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Binary fission, telotroch formation, pre-conjugation fission and conjugation are well-established phenomena in the subclass Peritrichida. Conjugation is the sexual method of reproduction in peritrichs, which not only differs strikingly from that of other ciliophorans in having a different number of progamic divisions, but also that the microconjugant becomes incorporated into the macroconjugant. It is also characterised by a sex-differentiating preconjugation fission that seems to occur in all families. Seventeen endemic limpet species occur between Cape Point and Cape Agulhas in the South African zoogeographic marine province. Limpets, represented by 3 genera, *i.e. Scutellastra* (H. and A. Adams, 1854) *Cellana* (H. Adams, 1869) and *Cymbula* (H. and A. Adams, 1854) were collected at the De Hoop Nature Reserve, taken to a field laboratory and dissected. Live symbiont specimens undergoing reproduction were observed with light microscopy. Gills were fixed in 10 % buffered, neutral formalin for SEM. In Bloemfontein, specimens were prepared for SEM using standard techniques. The nuclear apparatus was stained with haematoxylin and the infundibular detail was studied by staining Bouin's fixed smears with Protargol. Conjugation occurred frequently in all populations of *Mantoscyphidia branchi* (Van As, Basson and Van As, 1998). Preconjugants fuse and establish a cytoplasmic bridge between one another. The macronuclei disintegrate while the micronuclei undergo meiotic divisions to form pronuclei, which will fuse to form the synkaryon. The macro- and microconjugant separate and a series of divisions follow. The macronuclear anlage and the functional micronucleus arise from the division products of the synkaryon. The shrivelled and depleted microconjugant becomes detached and disappears, leaving only the macroconjugant (exconjugant). The swollen exconjugant returns to its vegetative state as the extra cytoplasm is evenly distributed during reorganisation.

Amphistomosis in Zimbabwe: epidemiological aspects

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Limited studies carried out on the highveld of Zimbabwe have shown that amphistomes were the most predominant parasites in the communal farming areas. Although amphistomosis has been diagnosed and treated in cattle in various parts of the country, little information is available on the epidemiology and transmission dynamics of amphistomes. The aim of this study was to determine the epidemiology and seasonal infection pattern of amphistomes in cattle and in the snail intermediate hosts in the highveld and lowveld communal farming areas of Zimbabwe. The study was carried out in the highveld and lowveld communal farming areas of Zimbabwe. Monthly rectal faecal samples were collected from cattle at randomly selected dipping sites in the highveld and lowveld communal farming areas. Faecal samples were quantitatively examined for amphistomes eggs and the results were analysed in relation to age, sex, location and season. Snail samples were collected at monthly intervals from the surrounding transmission sites. All snails collected at each site were screened for patent amphistome infections and percentages of snails infected with amphistome cercariae were calculated for each month at each site. The results revealed that 29.5 % of the animals were positive for amphistome eggs. The highveld had a significantly higher prevalence than the lowveld ($P < 0.0001$). There were highly significant differences in prevalence of amphistomes among the age categories ($P < 0.0001$), with adults having a higher prevalence than young animals. In both regions the prevalence was significantly higher during the wet season than the dry season ($P < 0.001$). The faecal egg output peaked from October to March. In total, 4082 *Bulinus tropicus* and 2535 *Biomphalaria pfeifferi* snail species were collected. *Bulinus forskalii* was relatively rare, with only 70 snail species collect during the 2-year period. *Bulinus tropicus* was significantly more common in the lowveld than the highveld ($P < 0.0001$). *Bulinus tropicus* breeds throughout the year reaching a peak from March to May while *Biomphalaria pfeifferi* peaked during the period January to March. Infection rates with amphistomes was 8.1 % in *Bulinus tropicus,* 1.4 % in *Bulinus forskalii* and 0.3 % in *Biomphalaria pfeifferi. Bulinus tropicus* started shedding amphistome cercariae in February, peaked during the period March to May, was low between June and July, peaked again between August and September and virtually no shedding occurred between October and January. Shedding by *B. forskalii* was only recorded in March 2000 and that by *Biomphalaria pfeifferi* in February, March and May 2000. Higher rainfall districts on the highveld had a significantly higher prevalence of amphistomes than the relatively drier districts in the lowveld. Prevalence and faecal egg output were higher during the wet season than the dry season. Adults had a significantly higher prevalence than young animals. *Bulinus tropicus* was found to be the main intermediate snail host. Both breeding and cercarial shedding by *Bulinus tropicus* peaked during the period March to May.

Myxozoans infecting the sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), in the Okavango River and Delta, Botswana

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The sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), is probably the most widely distributed fish species in Africa. As a result of its extensive use in aquaculture, the economic importance of this fish species has increased greatly in recent years. Furthermore, natural populations of *C. gariepinus* form a staple diet for many subsistence farmers throughout the African continent. Coinciding with the growing economic value of this fish is the increased interest in its parasite loads and what effect these might hold for the aquaculture industry. One particular group of parasites, the myxozoans are well known for the diseases they cause in commercially important fish hosts. In Africa, more than 135 species of myxozoans are known to infect freshwater, brackish and marine fishes. Six of these have been described from *Clarias* (Scopoli, 1777) hosts in Africa. During a recent investigation into the presence of myxosporeans infecting fishes in the Okavango Delta, Botswana, 14 *C. gariepinus* individuals were collected using a series of gill nets from lagoon environments within the Okavango Panhandle and Delta regions. Captured fish were

kept alive in aerated containers, anaesthetised with a dosage of benzocaine sufficient to kill them, measured and examined. Mature myxosporean spores were fixed in 10 % buffered neutral formalin, photographed with differential interference contrast and prepared for scanning electron microscopy using standard techniques. Tissue samples, fixed in Davidson's solution were prepared for histological sectioning using standard techniques. The results revealed the presence of 2 species from the genus *Henneguya* (Thélohan, 1895) and 1 species from the genus *Myxobolus* (Bütschli, 1882) infecting the *C. gariepinus* individuals. Large plasmodia of *Henneguya branchialis* (Ashmawy, Abu-Elwafa, Imam and El-Otifi, 1989) were found in the cartilage of the accessory-breathing organ, while plasmodia of a *Henneguya* sp. were found in the gills and a *Myxobolus* sp. was found infecting the ovaries.

Malaria control in South Africa and extension of control to southern Mozambique. Can we successfully keep malaria under control?

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South Africa has a long history of malaria control, starting in the 1940s. By the 1970s malaria had become a low risk problem in much of its former distribution in the country with the highest risk areas being border districts with Mozambique, Swaziland and Zimbabwe. The total number of malaria cases rarely exceeded 10 000 cases per annum prior to 1996. In 1996 however the total number of cases increased to approximately 20 000 and in 1999, more than 51 000 cases were reported, increasing again in 2000 to more than 64 000 cases. In 1999 insecticide resistance by the malaria vector mosquito *Anopheles funestus* and in 2000 drug resistance by the malaria parasite *Plasmodium falciparum* were detected in KwaZulu-Natal province. Appropriate policy changes in regard to insecticide and first line drug were instituted towards bringing transmission under control. Malaria vector control was extended to Maputo Province, Mozambique in 2000. Reductions in malaria cases as a result of these policy changes will be presented as will implications for the future control of the disease.

Acaricide resistance and cattle tick control in South Africa – the way forward

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Acaricide resistance in ticks, particularly *Boophilus* species, is a problem worldwide. Resistance mechanisms have evolved to all the major classes of acaricides presently used in *Boophilus* control. Continued uncontrolled use of these acaricides is selecting multiresistant populations in many countries. The tick resistance situation is therefore deteriorating and, with few new acaricides coming to market, there exists the potential for a serious breakdown in control of ticks, and hence of the diseases they transmit. Following reports of acaricide resistance in *Boophilus* species in South Africa a nationwide tick resistance survey was undertaken from 1998 to 2001 (L.J. Fourie (2001) National Tick Resistance Survey, unpublished report). Acaricide susceptibility bioassays using the major acaricide groups currently used in tick control in South Africa (pyrethroids, formamidines and organophosphorus) showed that resistance exists to all. However, few tick populations had individuals resistant to all acaricides. In order to prevent a further deterioration in the acaricide resistance situation in South Africa the selection and spread of acaricide resistance must be slowed. The only practical way of achieving this will be to institute a countrywide acaricide resistance management programme involving rotational use of acaricides. Such rotations have shown great promise in combating insecticide resistance selection in *Anopheles* vectors of malaria in Mexico. This presentation will detail the requisite steps in setting-up a resistance management programme and will discuss the potential problems in implementing such a programme in South Africa.

Investigation of a strategy for vaccination of free-ranging village chickens against Newcastle disease in Qwa-Qwa, South Africa

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In this study, antibody responses in free-ranging village chickens vaccinated with Nobilis ND Inkukhu thermostable Newcastle disease vaccine and corresponding protection levels against challenge with a local virulent field isolate were determined. Free-ranging village chickens at Thibella village were vaccinated 2 times in December 2001, and then once in March 2002. Blood samples were collected after 4 weeks of vaccination and every month thereafter until June 2002. Antibody responses were determined by haem-agglutination inhibition test. Three routes of vaccination were used *i.e.* eyedrop, drinking water and feed. The mean HI titres from January 2002 to June 2002 were 3.8 for eyedrops, 3.2 for drinking water, 2.6 for feed and 0.5 for controls. Challenge trials were conducted in July 2002 with 40 experimental chickens. All chickens were inoculated with 0.2 ml (1×10^6) of virulent Newcastle disease virus. All the control chickens died by day 3 and 4 post-challenge. Protection levels from eyedrop and drinking water were 70 % and 20 % from feed. *Post mortems* conducted on all the experimental chickens showed that vaccinated chickens had mild lesions while control chickens had severe lesions that were considered as Newcastle disease symptoms.

Aspects of the ecology of *Lamproglena clariae* from the Vaal River System, South Africa

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Lamproglena clariae, an ectoparasite of the freshwater fish *Clarias gariepinus,* was first described by Fryer in 1956. Previously, studies have been done on the taxonomy of *Lamproglena,* but little research has been published on its biology. This study aimed at determining and recording *L. clariae*'s ecology. Specimens were collected bi-monthly from the Vaal Dam and Vaal River Barrage in the Gauteng province for a 2-year period.The specific objectives of the study were to determine if there were any correlations between the size of the host and the size of parasite, the size of the host and the number of parasites; and the size of the parasite and the size of the gill filaments, as well as to determine if there was any significant difference between the number of parasites collected from male and female hosts. Fish specimens were weighed and measured in the field, while the size of the parasites was determined in the laboratory by using a light microscope with a micrometer.

The incidence, abundance and prevalence of the parasite were also determined. *Lamproglena clariae,* an ectoparasite of the freshwater fish *Clarias gariepinus* was first described by Fryer in 1956. Previously, studies have been done on the taxonomy of *Lamproglena,* but little research has been published on its biology. Hence, this study aimed at determining and recording *L. clariae*'s ecology. Specimens were collected from the Vaal Dam and Vaal River Barrage in the Gauteng province for a 2-year period.The specific objectives of the study were to determine if there were any correlations between the size of the host and the size of parasite, the size of the host and the number of parasites; and the size of the parasite and the size of the gill filaments, as well as to determine if there was any significant difference between the number of parasites collected from male and female hosts. Fish specimens were weighed and measured in the field, while the size of the parasites was determined in the laboratory by using a light microscope with a micrometer. The incidence, abundance and prevalence of the parasite were also determined. The results showed a positive correlation between the host's size and its parasites. Larger fish had both larger and more parasites than the smaller ones. Most of the parasites from both localities preferred the median arch of the 4th

gill for attachment. There was no significant host sex preference by parasites. The seasonal pattern was similar at both localities, with higher prevalence, abundance and mean intensity of *L. clariae* occurring during summer.

Blood parasites of reptiles from the Free State province South Africa

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South African herpetofauna is very diverse with 83 new reptile species described in just the past decade, which is 1 new species every 44 days. The Free State herpetofauna is unique mainly because of the variety of habitats, which ranges from the mountainous Afromontane to the Karoo. To date, there are 53 lizard species, 38 snake species, 1 amphisbaenian, 1 terrapin and 3 tortoise species described from the Free State. The aim of this study is to compile a survey of the blood parasites of these reptiles, as this will contribute to the systematics of the parasites. Practically no knowledge on these parasites exists in the Free State. Representatives of 7 families consisting of 20 genera and numerous species, mostly parasitic Protozoa, have been documented to occur in reptile blood worldwide. So far numerous parasites were found in reptiles collected. One of the more interesting parasites was an onchocercid nematode found in the peripheral blood of *Pseudocordylus melanotus*, *Cordylus polysonus* and *Agama atra.* Numerous haemogregarines were found in several species of reptiles, including a *Plasmodium* spp. that occurs in the red blood cells. Piroplasms of the genus *Pirhaemocyton* were found in abundance and these viral inclusions were present in all of the reptiles collected for this study. A number of parasites with uncertain taxonomic status were also found. All parasites found in the blood of these reptiles, are new host records, except infections of a viral nature found in *Agama atra* and *Mabuia capensis.* It is possible that most of the parasites collected are still undescribed species and thus broaden the knowledge of the diversity and nature of these organisms.

Branchiurians associated with fishes of the Okavango River and Delta, Botswana

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Fish were collected as part of a comprehensive survey of the fish parasite fauna of the Okavango River and Delta, using a variety of collection methods. In general 30 different ecto- and endoparasite genera have been identified, including representatives of the class Branchiura, *i.e. Dolops (*Audounin, 1837), *Argulus* (Müller, 1785) and *Chonopeltis* (Thiele, 1900). The distribution of *Dolops ranarum* (Stuhlmann, 1891), the only species of this genus found in sub-Saharan Africa is limited to the sympatric distribution of the clariid and cichlid hosts. The same applies to specimens found in the Okavango system, where males and females were collected from 2 catfish and 6 cichlid species. Only 2 species of the genus *Argulus* are known from southern African freshwaters, *i.e. Argulus capensis* (Barnard, 1955) and the introduced *A. japonicus* (Thiele, 1900) while 23 species are known from other African freshwater fishes. Of the 59 fish species examined, only the sharptooth catfish *Clarias gariepinus* (Burchell, 1822) hosted an *Argulus* species. The genus *Chonopeltis,* with 14 known species, is endemic to the African continent. Two of these species, *C. lisikili* (Van As and Van As, 1996) and *C. liversedgei* (Van As and Van As, 1999), were described from 4 different fish hosts, collected in the Okavango River and Delta. More than 80 fish species are known from this system and 15 of these fish species have so far been identified as branchiuran hosts. In none of these cases were more than 3 branchiuran specimens collected from a single fish host.

ABSTRACTS OF POSTERS

Lamproglena and *Lernaea* (Copepoda) as possible bio-indicators of environmental deterioration in the Olifants River

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The rising cost of water quality analysis and the persistent shortage of trained personnel necessitate consideration of alternative methods to routinely determine water quality. The Department of Water Affairs is in the process of developing biological indicators for this purpose. It is furthermore believed that biological indices are accurate not only at the time of sampling but also reflect on events prior to the sample being taken. Twenty specimens of *Clarias gariepinus* and *Oreochromis mossambicus* were collected bimonthly in 1994 from the Olifants River and examined for the presence of parasites. *Lamproglena clariae* and *Lernaea cyprinacea* were found to be present on the respective fish species. Initially, 2 river sampling sites were compared to one another and in a follow-up study in 1996, 2 sites from dams were added to the survey. The sites were selected to represent a polluted and a pristine environment. Serious floods occurred between the 2 sets of surveys. It was found that the prevalence as well as abundance of *L. clariae* and *L. cyprinacea* was higher in the pristine environment in the river localities in all 4 seasons. Following the floods both *L. clariae* and *L. cyprinacea* disappeared from the river localities, which were further downstream. In the dam with the pristine water quality, prevalence as well as abundance of *L. clariae* were higher than in the more polluted dam. *L. clariae* returned to the river sites approximately 1 year after the floods, and again the prevalence and abundance were higher at the site with the better water quality. *Lernaea* was not recorded after the floods. The effect of the flood was that the parasites were removed from the environment and re-infection by *Lamproglena* occurred only after the water quality returned to normal. The long-term effect of the flood was therefore illustrated. It is concluded that the use of these copepod parasites may be helpful in the development of a biological index for environmental integrity provided care is taken with the interpretation of results and consideration of biological variables such as temperature and host.

Reverse line blot: a diagnostic tool to detect blood parasites

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The Reverse line blot (RLB) is a versatile tool for the simultaneous detection and differentiation of blood parasites. It is based on the hybridisation of specific amplified DNA to oligonucleotides (probes). These probes are specific for the group of organisms of interest and will not hybridise to mammalian DNA. Samples of various animal species were obtained and blood was collected in citrate-, EDTA- and heparin-buffered tubes, and stored at –20 °C. Filter paper with blood spots was also collected and stored in a dry place at room temperature. Ticks fixed in 70 % ethanol and unfixed ticks were also collected. DNA was extracted from whole blood and ticks as described by Gubbels *et al.* (1999). DNA was also extracted from blood collected on filter paper using the FTA extraction reagent (Whatman Bioscience, Laboratory Specialist Services). PCR was performed on these samples and analysed using the RLB hybridisation technique as described by Gubbels *et al.* (1999). Probes are sensitive at genus (*Babesia* and *Theileria*) and species level.

Host	Specimen type	Specimens tested			Positive	
		Pos.	Neg.	Total	Genus	Species
African buffalo	Blood (EDTA)	437	525	962	437	437
Kudu	Unstained blood smears	19	0	19	19	16
Carnivore	Blood (citrate & EDTA)	4	0	4	4	3
Other	Cell culture, ticks, blood	66	12	77	66	21
Total		526	537	1063	526	477

The RLB technique is extremely versatile and represents a new research and diagnostic tool to detect and characterise blood parasites in animals and vectors (ticks). The results can lead to identification of new parasites and the information from a RLB can be applied in nano-technology techniques such as microarrays. This technique is used routinely in our labs for diagnostic tests.

The influence of antimalarial drugs on monocyte elemental concentrations as determined by X-ray microanalysis

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Each year over 200 million people succumb to malaria. The majority of the cases are in sub-Saharan Africa. In South Africa the number of malaria cases has risen steadily since 1992. This increase is due, in part, to the development of parasites resistant to chloroquine, pyrimethamine and other drugs. In the quest for new anti-malarial drugs a good understanding of mechanisms of action of current anti-malarials is vital. Monocytes play a central role in the orchestration of immunological reactions during malaria and other parasitic diseases. Monocytes secrete cytokines such as IL6, IL1 and TNF and have an increased secretion of interferon and neopterin during malaria infections. Monocytes are involved in the phagocytosis of malaria parasites. Anti-malarial drugs appear to influence monocyte secretions, phagocytic activity and down regulate the expression of monocyte receptors. Our approach has been to measure the composition of sodium, potassium, sulphur, chloride phosphorus and calcium in peripheral blood monocytes at time points from 10 minutes to 18 hours after exposure to a range of anti-malarial drugs. Elemental composition was determined using X-ray microanalysis. Monocytes were isolated from peripheral blood with density gradient centrifugation and adherence to plastic. Monocytes were then cultured on microscope grids and exposed to antimalarial drugs. Monocyte identity was confirmed with enzyme analysis. The results suggest that, unlike alterations in monocyte receptor expression and monocyte secretions which take up to 18 hours to alter in response to drug treatment, monocyte elemental composition are similar to controls in the presence of all anti-malarial drugs tested except quinine. Quinine alters calcium and potassium concentrations. Preliminary results suggest that changes in monocyte elemental composition occur during the first hour after exposure to anti-malarial drugs.

Species designation in the parasitic copepod *Nemesis lamna*

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Nemesis Risso, 1826 (Eudactylinidae: Siphonostomatoida: Copepoda), is parasitic on elasmobranch hosts. Differentiating between the *Nemesis* species on morphological grounds is difficult. Of particular interest has been the distinction of 2 subspecies, *N. lamna lamna* and *N. lamna vermi,* reported from different hosts. This distinction was made on a difference in the relative width of their free thoracic segments. Specimens of *Nemesis lamna* were collected from the great white shark (*Carcharodon carcharias*) and the shortfin mako shark (*Isurus oxyrinchus*). Morphological and molecular data are presented for the specimens collected from both hosts. DNA sequence data was collected from the *18S, COI,* and *16S* genes from 9 *Nemesis* individuals. Both maximum likelihood and Bayesian methods were used to reconstruct phylogenetic relationships. A scanning electron microscope was used to identify morphological characters differentiating the individuals from the 2 hosts and micrographs of the different appendages are provided. A sequence divergence of 1 % was found within the haplotypes of the *Nemesis lamna* clade and only a single distinguishing morphological character could be identified.

Nematode infections in dogs from peri-urban resource-limited communities in the Eastern Cape Province, South Africa

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The informal settlement areas around Grahamstown in the Eastern Cape Province of South Africa have limited access to veterinary services and regular de-worming of dogs in the area is therefore non-existent. The purpose of this study was to determine the species of nematode parasites in dogs from resource-limited communities in the area, particularly the zoonotic ones. In total, 652 individual faecal samples were collected from dogs and examined with a faecal flotation technique. Of these, 94 % were infected with hookworms, 51 % with *Trichuris vulpis,* 13 % with *Toxascaris leonina* and 9 % with *Toxocara canis.* Except for *T. vulpis,* all other nematodes reported in this study are zoonotic, and therefore pose a threat to community health in the Grahamstown area.

A role for peripheral blood fibrocytes in African trypanosomiasis and Lyme disease

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It is proposed that peripheral blood fibrocytes (PBFs) play an important role in the pathogenesis of African trypanosomiasis and Lyme disease. The PBF represents a leukocyte subpopulation that (a) expresses collagen, (b) is an abundant source of cytokines, chemo-attractants and growth factors, and (c) is able to recruit and activate naïve T-cells and/or memory T-cells. Uninfected C57BL mice, experimental animals resistant to infection by African trypanosomes and the spirochaete causing Lyme disease, *Borrelia burgdorferi,* have significantly less PBFs in peripheral blood than do disease sensitive C3H mice. During an acute *B. burgdorferi* or *Trypanosoma brucei* infection the number of PBFs were found to increase in the C3H animals which contrasted to the decrease in PBF number in the C57BL mice. It is speculated that resistance to *T. brucei* or *B. burgdorferi* shown by C57BL mice may be related to initial high PBF levels. Preliminary evidence also suggests that *Borrelia*-pulsed PBFs can prime naïve T-cells *in vivo.* PBFs or macrophages (Mø) obtained from C3H mice were pulsed *in vitro* with fixed *Borrelia* and then injected intradermally into the rear foot pad of unprimed C3H mice. Ten days later the draining lymph nodes (DLN) were removed. The proliferative response of the constituent cells was tested alone or in the presence of spirochaetes or non-relevant antigens (*e.g.* ovalbumin). *Borrelia*-pulsed PBFs were found to induce a significant T-cell proliferative response. This response was specific for the priming antigen (formalin-fixed spirochaetes), as the response of DLN to ovalbumin was low, similar to DLN cells alone. The priming of naïve T-cells in the DLN by PBFs suggests the migration of *Borrelia* antigen-pulsed PBFs into this organ and that the degree of priming of T-cells in vivo by PBF was superior to that induced by Mø.

Micromorphology of the Kiwi feather louse (*Rallicola gracilentus*)

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Rallicola gracilentus is a host-specific ectoparasite of the great spotted kiwi (*Apteryx haastii*), a threatened species in New Zealand. The chewing mouthparts of these lice are used to ingest feathers and epidermal debris and, as with poultry lice, heavy infestations may stunt the growth of the young birds. The micromorphological specialisations of this louse were investigated by scanning electron microscopy (SEM). Lice collected from great spotted kiwi in the Mt Cook National Park, New Zealand, were fixed in 70 % ethanol. After cleaning by ultra-sonication in detergent, the specimens were

routinely processed for SEM and viewed in a Leica Stereoscan 420 SEM at 5–7 kV. The SEM study revealed several specialisations not clearly visible on traditional slide-mounted specimens. On the ventral surface of the broad shovel-shaped head, the lateral folds of the carina forms a deep medial groove that contains the membranous pulvinus that surrounds the oral cavity. The feather barbules are pushed into this groove by the mandibles when the louse attaches to the feathers. The mandibles frequently has double-notched cutting edges for feeding by scraping the host's epidermis. A pair of labial palps, each bearing 6 terminal peg-like sensory sensilla, lie just posterior to the mandibles. Several specialised sensoria were observed on the antennae. The terminal peg organ consists of 12 sensilla of varying lengths. Two sensory pore organs and their associated plate organs on the 4th and 5th segments are reported here in *R. gracilentus* for the first time. Each pore organ contains a tuft organ as well as an adjacent plate organ with radiating slits. These sensoria are reported to be chemosensory, enabling the lice to orientate on their host. The luminal surfaces of both the abdominal spiracles and the larger thoracic spiracles are lined by irregular lamellae that may function to filter the air passing through the spiracles into the tracheae. Each leg has 2 terminal tarsal claws. The larger anterior curved claw closes between the 3 opposing conical setae of the pretarsal sclerites to firmly grasp the barbules of the feather. The 2nd, slightly curved claw remains open at an angle to the large claw to easily grasp adjacent barbules. The 3 spinous setae on each of the paired tubercles on the terminal gonopods of the female confirms the identification of this louse as *R. gracilentus* according to Clay (1972). In this study the everted aedeagus with its laterally directed, leaf-shaped parameres, which protrude from the genital opening of some of the males, was observed for the first time.

Tissue-burrowing copepod parasites of *Hilsa kelee*

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The Pennellidae represents a peculiar family of siphonostome copepods that are known almost exclusively from female specimens. This is due to the fact that an intermediate host is involved in their life cycles and the adult males die soon after mating while they are on or in the intermediate host. The post-mated adult females leave the intermediate host, seek out a final teleost host, bore into the tissues or organs of the host, and leave only the tremendously enlarged and/or elongated genital and abdominal portions of the body exposed. The family is known from these mesoparasitic adult females on the teleosts, the ephemeral males having seldom been reported. *Lernaeenicus* sp. was found on 5 *Hilsa kelee* fish hosts collected during fish surveys carried out in the Mhlatuzi Estuary in Richards Bay on the east coast of South Africa. All the hosts with copepods were fixed in 10 % formalin, and later transferred to 70 % ethanol. The copepods were carefully dissected out of the tissue of the hosts and placed in 70 % ethanol. The site of insertion is usually variable, but all the copepods found in the present study were found inserted just beneath the pectoral fin, with the head buried in the host's tissue, never hanging free in the host's body cavity. The trunk of the parasite is not wrapped by a 'host tissue band', as is seen in some other pennelid genera. Nominal species abound in *Lernaeenicus*, many of which have been very poorly described. Some are known only from single records. A thorough revision of the genus is badly needed to piece the life history of these burrowing parasitic copepods together. The parasite represents a new host and distribution record for South Africa.

Skin and gill parasites of the Kob, *Argyrosomus japonicus* (Temminck & Schlegel, 1843) collected at the De Hoop Nature Reserve, South Africa

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The kob, *Argyrosomus japonicus*, is 1 of the best known and widely spread sport fishes in southern Africa and is also commercially exploited by line-boat fisherman. This shoaling predator occurs at depths of up to 400 metres and is also common in shallower coastal areas, particularly along the sandy edges of reefs and estuaries where the water is often turbid. During a recent investigation of the parasites of surf zone fishes at the De Hoop Nature Reserve, 4 kob were collected by means of rod and line. Fish were identified, measured and examined for parasites. Ecto- and endoparasites found were removed and fixed according to standard methods required for each specific group. The genus *Caligus* (Copepoda) was found abundantly on the skin, with prevalence of 100 % and up to 8 specimens on a single fish host. Three different parasites, *i.e.* 2 copepods from the genera *Neobrachiella* and *Sciaenophilus* and 1 unique monogenean from the genus *Benedenia* were found in the branchial areas. Another monogenean of the genus *Udonella* was found associated not with the kob, but with the caligid copepod. These hypersymbionts feed mainly on the epithelial tissue of the fish host and do not cause any harm to the copepod host.

Applying the parasite index (PI) as a bio-indicator of water quality in the Selati River, Limpopo Province: preliminary results

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Water quality monitoring in South Africa has in the past focused mainly on measuring physical and chemical variables. The value thereof is well established, but these methods cannot on their own provide an accurate measure of the general 'health' of an aquatic ecosystem. Subsequently the fish Health Assessment Index (HAI) and associated Parasite Index (PI) have been applied and adapted for local conditions through various studies in the Olifants and Vaal River Systems. The HAI proved to be a relatively rapid and inexpensive method to detect changes in a fish population. These studies also showed that the presence of parasites *per se* was an indicator of the deteriorated health of the fish and consequently a deteriorated environment and that fish parasites are extremely sensitive to changes in the aquatic environment. The Ga-Selati River, which originates in the Drakensberg Mountain, was selected for this study owing to the mining activities on its banks before its confluence with the Olifants River. Four sampling sites in the lower Selati River were selected: 3 sites in the vicinity of the mines and 1 site representing an 'unpolluted' part of the river, 30 km upstream from the mines. Thus far 2 seasonal surveys have been conducted which included the water quality constituents and the HAI and PI. Two species of fish, *Oreochromis mossambicus* and *Clarias gariepinus*, were collected by gill netting and angling. Hosts were examined for mobile ectoparasites and dissected to examine internal organs using the revised HAI method. All parasites collected were fixed and preserved using standard methods. Preliminary results indicate that the water quality is very poor at the sampling sites at the mines, with a very high salinity and conductivity caused by the calcium, sodium, potassium and magnesium salts. The sampling site upstream from the mines, with a much better water quality, showed lower TDS values. The PI correlates with these findings, with more ectoparasites being present at this sampling site and more endoparasites at the mining sites where water quality was poor. The use of parasites in the HAI, or individually as indicators of pollution (PI), has potential and should be applied in more water bodies of South Africa.

New information on the location of Bruce's laboratory at Ubombo as a basis for possible archaeological studies

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By means of recent surveys, the site where *Trypanosoma brucei brucei* was discovered in 1895 by Major General Sir David Bruce (KCB, DSc, LLD, FRCP, FRS, Late AMS), could be determined with more accuracy. A GPS reading of S 27° 33.681' and 32° 05.114' can

now be accepted as the location of the site with a high degree of certainty. Comparing the background of the present site with the background as seen in photographs taken during Bruce's stay, a better match is obtained than in the previous site. Remains of structures present on the site could shed more light on the conditions if research is undertaken in the future. It is still uncertain whether the new site falls within the borders of the plots allocated to PARSA. Two camps where experimental animals were kept, and which Bruce referred to as 'camp in the thorns' have been located. The 1st, Nkonkeni, was 10 km to west-nortwest, while the 2nd, nDelakufa, was located 11 km to the east-southeast of the base camp on the plateau. No permanent structures were erected at these sites which today are situated on ground belonging to (1) Senekal Boerdery and (2) KwaZulu-Natal Natural Resources. Access to the camp by wagon was possible only by the now defunct old wagon trail from Mkuze. Mapping the area is hampered by the dense alien vegetation that now patchily dominates the grassland on the slope. Further verification of the findings is being done by A Van der Venter who is the Senior Archaeologist with Amafa, which is responsible for the heritage of KZN. After further analysis they will decide whether this site should be a national or provincial heritage site.

Incidence of *Cryptosporidium* at Groote Schuur Hospital

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Cryptosporidium is a minute coccidian parasite that causes self-limiting diarrhoea, usually in young children and immunocompromised people. The infective stage of *Cryptosporidium* is an oocyst containing sporozoites. The development of sporozoites occurs within the brush border of epithelial cells of the intestine. Sporulated oocysts that contain 4 sporozoites each are passed in the stools of infected patients. Using an immunofluorescent staining technique (auramine), these are visualised as small round bodies, 2–4 μm in size. In the diagnostic microbiology laboratory, all stool specimens from children under 6 years of age are routinely examined for the presence of *Cryptosporidium* oocyts. Stool specimens from patients 6 and over are only screened for *Cryptosporidium* at the request of the clinician or when clinical data indicates that the patient is immunocompromised. Data collected for a period of 1 year (2001) revealed the following results: Of the 183 specimens examined in children under the age of 6, 21 were positive (11.5 %). Of the 227 specimens examined in immunocompromised persons 6 and over, 14 were positive (6.2 %). Of the 21 positives found in children under 6, only 1 occurred in a child older than 1 year.

These results reflect a high incidence of *Cryptosporidium* in children 1 year and younger, and suggests that routine screening in children above this age is not indicated. The relatively low incidence of *Cryptosporidium* in selected patients 6 years and older suggests that the selection criteria may need to be changed in order to yield a more cost-effective result.

A new cestode from *Marcusenius macrolepidotus* from Mpumalanga

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During a parasitological survey of mormyrid fishes in the Limpopo and Mpumalanga provinces, an interesting cestode was found in the intestine of *Marcusenius macrolepidotus.* This cestode could not be assigned to any known genus within the Proteocephalidae. Cestodes were cleaned in 0.8 % saline solution and relaxed by swirling them in a small amount of water in a sample bottle. After muscle fatigue set in, specimens were fixed by adding hot AFA and preserved in 70 % ethanol. Wholemounts were stained in Horen's trichrome stain, counterstained in acetocarmine and mounted in Canada balsam. Serial sections were made using standard techniques and stained with Delafield's haematoxylin and counterstained with aqueous eosin. Conventional methods were used for scanning electron microscopy. The Proteocephalidae is primarily characterised by medullary vitelline follicles and gonads, *i.e.* internal to the longitudinal muscle bundles, while the proteocephalid subfamilies are differentiated by the characteristics of the scolex, *i.e.* the presence or absence of the metascolex, armed rostellum, piercing organ and spines on the scolex. This cestode has some characteristics of the genera *Gangesia* (Woodland, 1924) and *Vermaia* (Nybelin, 1942). The following characteristics are of importance: the scolex bears a protrusible rostellum with a single row of large hooks of unequal size, rostellar hooks are blade-shaped and arranged in a concentric circle or crown at the distal end of the scolex, twenty large and twenty smaller hooks are present as are 4 large suckers without spines or spinelets, the scolex, neck and strobila are devoid of cuticular spines, a distinct unsegmented slender neck is present and the genital pore alternates irregularly. This cestode appears to be related to *Gangesia,* a genus characterised by the presence of cuticular spines on the suckers. The new cestode, however, is devoid of cuticular spines and further histomorphological studies are necessary to determine its true identity.

Epitope mapping of a trypanosomal cysteine proteinase

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Trypanosomosis is a parasitic disease of man, domestic and wild animals and is of major economic importance in many parts of the world, particularly in sub-Saharan Africa. The parasite itself is not directly responsible for the disease, but rather causes illness through the release of pathogenic factors. One of the major pathogenic factors secreted by trypanosomes is a proteinase. The trypanosomal proteinases could be inhibited by antibodies and the formation of antibodies could be induced in cattle by immunisation with trypanosomal proteinases. The peptides from the catalytic domain and C-terminal extension of a trypanosomal cysteine proteinase, congopain, were selected using an epitope prediction program. Peptides selected were from the 2 forms of congopain called CP1 and CP2. M-maleimidobenzoyl-N-hydroxysuccinimide ester or glutaraldehyde was used to conjugate peptides to rabbit albumin. Antibodies against peptide-carrier conjugates were produced in chickens. The antibodies recognised native congopain, recombinant CP2 and the catalytic domain (C2) as shown by ELISA tests and western blots. This indicates that the peptides selected have promise for use in vaccines. The peptides were also used to determine whether they are natural immunogenic epitopes of CP2. Antibodies in the sera from *T. congolense*-infected cattle recognised all the peptides in an ELISA. Antibodies in the sera from C2-immunised cattle (non-infected) recognised most of the peptides in an ELISA. These tests showed that most of the peptides could be used in diagnostic assays. In order to distinguish between *T. congolense* and *T. vivax* infection, 2 different peptides from the C-terminal extensions of CP2 and vivapain were used in ELISA tests with sera from infected cattle. We report on the results of these studies identifying congopain epitopes that could be used in vaccines and diagnostic assays.

Some notes on the cestode larvae (Cestoda: Tetraphyllidea) found in the alimentary canal of *Octopus vulgaris* from the De Hoop Nature Reserve, South Africa

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Adult stages of tetraphyllidean cestodes are exclusive parasites of the spiral valves in the intestines of elasmobranchs and holocephalids, thus being exclusively marine. Representatives of the Tetraphyllidea (Carus, 1863) are often regarded as the most primitive group of tapeworms. Tetraphyllidean larvae have been reported to occur in octopods, marine teleosts and marine mammals. The life cycles of these organisms are mostly unknown. Larvae change quite a bit during development into adults, making identification difficult. The scolex is the most complex and diverse amongst the tapeworms. Taxonomy of this group is almost solely

based on the morphology of the scolex. It has 4 attachment organs called bothridia or phyllidea (lappet/leaf-like outgrowths). These structures are thin with flexible margins, which may be extremely variable, very mobile, stalked or sessile. The bothridia sometimes have hooks, spines or suckers. *Octopus vulgaris* was collected during low-tide on the rocky shores of the De Hoop Nature Reserve on the south coast. The octopus was dissected and the intestines were examined under a compound microscope. Live tetraphyllidean larvae were found in the intestines, moving around by flapping the bothridia. Some specimens were fixed in 70 % ethyl alcohol for SEM, while others were fixed flat for light microscopy. In the laboratory in Bloemfontein the specimens were prepared for SEM using standard techniques and photomicrographs were taken to measure body dimensions. The larvae were identified as plerocercoid larvae possessing an adult scolex, but lacking the embryonic hooks of the procercoid from which it developed. The larvae belong to the family Onchobothriidae (Braun, 1900). It has underdeveloped hooks and probably belongs to the genus *Calliobothrium* (Van Beneden, 1850) since the bothridia are divided by 2 transverse septa into 3 loculi or areolae.

A study on quantitative descriptors in the ecology of a *Diplozoon* sp. *on Labeo umbratus* in the Vaal River

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To date, little research into the order Monogenea has been conducted. Details of the ecology of these organisms are vague at best. To address this, live *Diplozoon* specimens were collected from the gills of *Labeo umbratus* in the Vaal Dam and Vaal River Barrage systems in Gauteng. The fish were collected using gill nets with mesh sizes of 90, 110 and 130 mm, respectively, over a 2-year period, every 3 to 4 months. During collection of the parasites, host data recorded included total length, fork length, weight and sex, and these were analysed statistically using the SPSS. The following quantitative descriptors were determined; prevalence, mean intensity, incidence, condition factor, and correlations regarding preference for host size and sex. Further investigations were made into the preference for attachment site of the parasites. The seasonality of the parasite infestations was also analysed. The statistical results analysis indicated that prevalence, mean intensity and incidence fluctuated over seasons. All values were lower during the colder winter months than in summer months when water temperature is higher and co-dependant adults are present in greater numbers. A higher mean intensity was recorded on larger fish, owing to the obvious lack of habitat restrictions, and on male fish. This could be due to the unique spawning habits of *Labeo* spp., where a number of males congregate around single females. Analysis of the condition factor showed a negative correlation between intensity of parasitism and the condition factor of the fish. This indicates that the presence of the parasites does have a negative effect on the well being of the host. Attachment site preference has been a point of controversy in the past. In this study it has been shown that no preference exists between attachment to left or right gill areas, or to attachment on the 1st, 2nd, 3rd or 4th gill arches on either side.

Identification of a *Plasmodium falciparum* protein with sequence similarity to a protein that protects mice from a *Plasmodium chabaudi* infection

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There are over 200 million cases of malaria each year and some 2 million fatalities. Most of the fatalities are due to the *Plasmodium falciparum* malaria parasite. In South Africa the number of malaria cases has risen from 600 in 1992 to over 27 000 in 1999. The rise in malaria cases is due, in part, to the development of drug-resistant parasites. There is an urgent need to develop a malaria vaccine. We have used a mouse malaria model to search for protective mouse malaria antigens with vaccine potential and have identified a malarial antigen (Pc96) that protects mice against a *Plasmodium chabaudi* infection. The *Pc96* gene was obtained from a *lambda* gt11 expression library using monoclonal antibodies raised against the purified Pc96 protein. The recombinant protein and a protein fractionated by size and ion-exchange chromatography and identified by the monoclonal antibodies stimulated the proliferation of a T-cell clone. The T-cell clone was previously shown to protect athymic nude mice from a lethal *P. chabaudi adami* infection. The *Pc 96* gene was cloned and sequenced and the deduced amino-acid sequence used to screen the *Plasmodium falciparum* genomic data base. An amino acid sequence of a 386kDa protein with regions of sequence similarity to the Pc96 gene was identified. The *Pc96* and *Pf386* sequences have a region of 90 amino acids with a high degree of sequence similarity. The region contains a number of predicted T-cell and B-cell epitopes. A series of overlapping portions of the *Pf386* gene will be amplified by the polymerase chain reaction from a sample of falciparum genomic DNA and cloned into an expression vector. The 1st of the series of amplified regions has been cloned into an expression system for screening by antibodies and purification of the expressed protein for evaluation.

Prevalence of helminthic infections in a survey of adults in the Durban area

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More than a billion people worldwide are believed to be infected with *Ascaris lumbricoides,* 500 million with *Trichuris trichiura,* 900 million with hookworms and in excess of 200 million with schistosomes (Cooper and Bundy 1987; Compton 1989). This paper extrapolates information from the 1st visit of a 5-year family study carried out by the Amoebiasis Programme of the MRC in collaboration with the University of Minnesota, Minneapolis, on the close associates of 91 patients with amoebic liver abscesses. A total of 795 stools were collected and formol-ether concentrations of the stools microscopically examined. Statistical analyses were performed on demographic data and microscopy results. Health professionals are realising with increasing frequently the detrimental impact that parasitic diseases have upon the lives of rural communities and the far-reaching sequelae. Most previous studies have been conducted on school-going children. This study was performed on adults; children were excluded. The parasitological results and the demographic data will be presented.

Epidemiology of *Toxocara canis, T. cati* and *Toxascaris leonina* in Jimma, southwestern Ethiopia

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Toxocara canis, Toxocara cati and *Toxascaris leonina* are roundworms of dogs and/or cats. The study was conducted to determine the epidemiology of these parasites and other intestinal helminths of dogs and cats in the Jimma region of Ethiopia. In a cross-sectional study the following samples were investigated using a saturated sugar flotation method: 430 faecal droppings of dogs from residential areas, roadsides, open fields, gardens and playgrounds; 230 faecal samples of dogs taken from the rectum; 77 faecal samples of cats from households and residential areas and 242 soil samples from residential areas, roadsides, open fields, gardens, playgrounds, house dust and dust of dog houses. It was found that 60.78 % of faecal samples of dogs from the environment, 78.60 % of faecal samples of dogs taken from the rectum, 66.23 % of faecal samples of cats and 33.38 % of soil samples were positive for 1 or more parasites. Parasite species identified were *T. canis, T. cati, T. leonina,* hookworms, *Spirocerca lupi, Trichuris vulpis,* taenids, *Ascaris* species and/or *Strongyloides* species. A relatively high mean egg count of 15.25 with a range of 2–67 was observed for *T. canis* in faecal samples of dogs collected from the environment. This study has indicated the presence of *T. canis, T. cati, T. leonina* and other helminths in faecal samples of

dogs and cats, and soil samples in the study area. As most of the dogs are free-roaming and randomly contaminate the environment with their faeces, combined with the present low standard of living and hygiene in the country, a high risk of zoonotic infections is present.

Transplacental and transmammary modes of transmission of *Toxocara canis*, *Toxocara cati* and *Toxascaris leonina* in a paratenic host

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Transplacental infection with *Toxocara canis* and transmammary infection with *Toxocara cati* are the major routes of transmission in dogs and cats, respectively. Rodents, humans and other animals may act as paratenic hosts. The aims of this study were to determine the possible transplacental and transmammary transmissions of these parasites and *Toxascaris leonina* in mice and if transmission occurred, to determine whether it is from infection acquired before or during pregnancy. Thirty-four female mice were infected prior to and 18 during pregnancy. The pregnant mice, before or after giving birth, their foetuses and newborns were dissected. Tissues and organs were digested using artificial gastric juice. Larvae were recovered using a Baerman apparatus. Larval burdens were determined. In mice infected before pregnancy and dissected before or after giving birth, no larvae were found in foetuses, placenta or uterus. Larvae of *Toxocara canis* and *Toxocara cati* were recovered from newborns and mammary tissues. In mice infected during pregnancy and dissected before or after giving birth, larvae of *Toxocara canis* were recovered from the placenta, uteri, mammary tissues and newborns. In mice infected with *Toxocara cati* larvae were recovered in newborns and mammary tissues, none in the placenta, uteri or foetuses. None of the mice infected with *Toxascaris leonina* had larvae in the placenta, uteri, mammary tissues, foetuses or newborns. This study has indicated the possible transplacental transmission in mice infected with *Toxocara canis* during pregnancy, and possible transmammary transmission when infected before or during pregnancy. Although possible transmammary transmission of *Toxocara cati* from infections acquired before or during pregnancy was observed, there was no evidence for transplacental transmission. Neither transplacental nor transmammary transmission was observed in mice infected with *Toxascaris leonina.*