

Parasitological Society of Southern Africa

The following are abstracts of papers and posters presented at the 34th Annual Congress of the Parasitological Society of Southern Africa (PARSA), 25–28 September 2005, Magoebaskloof Hotel, Magoebaskloof.

Keynote Addresses

The usefulness of parasites

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Parasites are notoriously hated for their presence in society and the effect they have on animals. Their economical value is usually expressed in negative numbers to explain the loss of energy or profit they cause. There is, however, another angle to the presence of parasites. Leeches are frequently used, even in modern medicine, after microsurgery as they possess a unique substance which is injected when feeding to prevent host blood clotting in their proboscis. This substance fulfils the same function in humans after microsurgery and speeds up recovery or prevents gangrene. Parasites also have an effect on the development of healthy resistant blood lines by eliminating the weakest individuals in a population. Indiscriminate or illegal introduction of hosts will invariably bring a spectrum of parasites along. When the parasites are either species- or location-specific, this information is used to trace the origin of the host. They are therefore useful as indicators for translocations. In extremely species specific monogenean parasites they are even used as identification tools for host species. In salmon the movement of individual brood stocks are traced across oceans with their parasites acting as the tags. It is even possible to determine whether these fish move back to the freshwater stream where they hatched by studying the parasites they acquired as juveniles, before moving to the ocean where they reached adulthood. During the last number of years parasites have also been shown to be useful as indicators of environmental deterioration. Various authors have shown that they are useful as indicators at the community level and many other authors have shown their use at a species level. In a review these topics are discussed.

Polystomatid biogeography and evolution

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Monogenean flatworms are mainly parasitic in lower aquatic vertebrates, including fish, anurans and chelonians. The parasite family Polystomatidae is represented by 20 genera. *Diplorchis*, *Eupolystoma*, *Mesopolystoma*, *Metapolystoma*, *Neodiplorchis*, *Parapolystoma*, *Parapseudopolystoma*, *Polystoma*, *Protopolystoma*, *Pseudodiplorchis*, *Riojatrema*, *Sundapolystoma* and *Wetapolystoma* are known from anuran hosts, *Pseudopolystoma* and *Sphyrnanura* from urodelid hosts, *Neopolystoma*, *Polystomoidella* and *Polystomoides* from chelonians, *Concinocotyle* from the Australian lungfish and *Oculotrema* from a mammal, the hippopotamus. While some genera have a worldwide distribution, others are restricted to small geographic regions. Of the presently known 153 polystome species, no less than 61 % are known from anuran hosts while 3 % are known from urodelid amphibians. Until now the Gymnophiona or Apoda was not known to harbour any polystomes. This paper also reports on the 1st finding of polystomes from the urinary bladders of *Caecilia* cf. *pachynema* and *C.* cf. *gracilis* from Ecuador and Peru, respectively. The parasites are small with a maximum body length of 2mm. The 2-gut caecae are not confluent posteriorly with any diverticulae or anastomoses. The haptor has 6 well-developed suckers and 1 pair of hamuli. A single follicular testis occurs in mid-body; the ovary is small; vaginae are present, and a single large operculated egg occurs in a short uterus leading to the armed genital bulb. The larval stage and phylogenetic position are not known.

Fish parasites as potential pollution indicators

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There are 3 different hierarchical levels at which parasites could be investigated as potential biological indicators in relation to environmental stress; 1st, at the level of an individual; 2nd, the level of a population and 3rd, the level of a community. The examination of attachment apparatus of selected parasites revealed abnormal morphological changes. As a result of various types of environmental pollution, a decrease in parasite species richness, as well as an increase of homogeneity of parasite distribution within the parasite component community was also recorded. Based on parasite species richness and heterogeneity measures, the fractionation of the parasite component community appeared to be ecologically reasonable. Relationships between the composition and structure of parasite infracommunity with respect to the level of chemical contamination and biochemical markers of exposure in individual fish were found. It is evident from detailed analysis of hierarchically structured data that parasites of fish seem to be a perspective nonspecific indicator of freshwater ecosystem integrity.

Milestones in South African malaria control

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An account of how local and overseas findings have influenced malaria control during the past century is given with particular reference to the original Transvaal region. De Meillon, Annecke and Park Ross are among those who initiated the change from severe malaria epidemics to an acceptable level enabling economic development and minimum mortality. The introduction of residual insecticide for indoor house application was the turning point. Since the 1960's the climatic variables, resistance to drugs and insecticides, as well as social changes have been moderated by the introduction of appropriate measures. Provided continuous assessment of malaria transmission is made and control measures promptly adapted to changing epidemiology, the infection can be confined to low levels. Episodes are described to illustrate personalities and events during this period.

Helminth parasites of African game animals

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A large scale cull of game animals was carried out in Kenya by the Food and Agriculture Organization of the United Nations. The justification and the procedures followed for this project are presented. Parasitological examination of culled animals was carried out. The methods followed for this, mainly helminthological examination, are presented. Some of the helminth parasites encountered and their pathological effects are identified and illustrated. The parasites recovered from 555 wildebeests, *Connochaetes taurinus*, their incidences and intensity of infections are listed and tabulated. The total worm burdens of these wildebeests are provided and analysed with regard to age, sex and immunological condition of the hosts.

Oral Presentations

Identification and characterisation of *Theileria lestoquardi* antigenic proteins

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The diagnosis of *Theileria lestoquardi* infection is mainly based on the clinical signs and demonstration of the schizont stage in lymph nodes or organ biopsy smears. An indirect fluorescent antibody tests (IFAT) to diagnose *T. lestoquardi* has been described. However, there are doubts about the specificity of the test based on crude schizont antigen and, hence, its suitability for epidemiological surveys in areas where more than 1 sheep *Theileria* pathogen could possibly exist is controversial. The aim of this study was to identify antigenic proteins of *T. lestoquardi* suitable for the development of a recombinant protein-based ELISA. One strategy was to search for homologue proteins previously identified in other *Theileria* species. Thus, cDNA of the *T. lestoquardi* homologous gene to the *T. annulata* surface protein (*TaSP*) was cloned and recombinantly expressed. Western blot experiments revealed high specific reactivity of sera collected from *T. lestoquardi*-infected sheep against this protein, making it a suitable candidate for ELISA. Furthermore, the homologue of the *T. parva* P32 gene was completely sequenced and its immunoreactivity is under evaluation. Further strategies included random-sequencing of a *T. lestoquardi* schizont cDNA bank with the subsequent bioinformatic analysis and screening of the cDNA bank with serum from infected sheep. Using the latter approach, a new antigenic parasite protein was identified and an ELISA is currently being validated.

In vitro ovicidal and larvicidal activity of *Peltophorum africanum* extracts against parasitic nematodes of small ruminants

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Parasitic nematodes are a major cause of production losses arising from depressed milk, meat and wool yield, and stock mortality in small ruminants. Although synthetic anthelmintic drugs are used to control helminthosis, widespread use and misuse has resulted in multiple anthelmintic resistance, necessitating a search for alternative ways of parasitic control. In developing countries, 80 % of the populations depend on herbs for primary health care in humans and animals. However, there is a need to validate the efficacy of phytomedicines before their acceptance and use worldwide. The objective of the study was to determine *in vitro* efficacy of acetone extracts of *Peltophorum africanum* against important livestock parasitic nematodes, *Haemonchus contortus* and *Trichostrongylus colubriformis*. Extracts of the leaf, bark and root were incubated with the eggs and larval stage (L₁) for 2 and 5 days, respectively. Thiabendazole[®] and water were positive and negative controls, respectively. Concentrations of 0.2–1.0 mg/ml of the leaf, bark and root extracts inhibited egg hatching and development of L₁ to the infective stage (L₃). At concentrations of 5–25 mg/ml, complete lyses of the eggs and L₁ of both *H. contortus* and *T. colubriformis* occurred. The *in vitro* model demonstrated the anthelmintic effects of *Peltophorum africanum* extracts. Work continues to establish the mode of action of the compounds in the extracts. The ongoing work may lead, not only to possible isolation of novel anthelmintics from the plant, but also to better methods of plant extraction readily adaptable for use by rural communities against helminthosis.

Scanning electron microscopical observations on the louse *Seamundssonina lari* from the grey-headed gull

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An extensive literature search revealed that no scanning electron microscopical investigation has, to date, been performed on *Seamundssonina lari*, a mallophagean louse feeding parasitically on the feathers of many gull species. Live lice were collected from Grey-headed gulls that congregate on a landfill site near Johannesburg. The specimens were fixed in 70 % ethanol and identified. The lice were routinely prepared for scanning electron microscopy and viewed in a Leica Stereoscan 420. The SEM investigation revealed several micromorphological features which confirmed the identification of the lice. Previously unrecorded features were also noted. The head corresponded to that of the typical philopterid genus *Seamundssonina*. Prominent characteristics on the dorsal surface included a broad hyaline margin on the ante-clypeus, the dorsal anterior plate showed a thickened, pointed anterior margin, 2 prominent lateral pre-antennal trabeculae and a broad post-antennal suture. Ventrally the left mandible revealed the typical toothed molar lobe characteristic of *Seamundssonina*. Peg organs comprising 10 sensilla occurred on the antennal tips. The thorax had 2 lateral, oval-shaped mesothoracic spiracles. Each leg bore 2 pretarsal claws that were apposed by 3 robust pretarsal setae. Six pairs of abdominal spiracles were present on segments III–VIII, whilst the 2nd segment only showed a stigmatal scar with no spiracle. Caudally directed, laterally positioned, pointed processes were recorded on segments I and II.

Prevalence, distribution and possible vector of *Hepatozoon pettiti*, blood parasite of Nile crocodiles in the Okavango Delta, Botswana

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Haemogregarines of the genus *Hepatozoon* have been reported infecting the erythrocytes of Nile crocodiles (*Crocodylus niloticus*) from various regions in Africa, including the Okavango Delta, Botswana. Since December 2001 members of the Okavango Crocodile Project captured crocodiles by means of nooses, Pitman traps and box traps, in order to study their ecology and physiology. Blood smears from 186 crocodiles were made to investigate the distribution, pathology and possible vector of *Hepatozoon pettiti* in the Okavango Delta. Ectoparasites of crocodiles were also collected. The only ectoparasite found was the leech *Placobdelloides multistriatus*, infesting 17 of the 186 crocodiles. Blood slides were air dried, fixed for 5 minutes in methanol, stained for 20 minutes in Giemsa's stain and analysed and photographed using a Zeiss Axioplan light microscope and Sony photomicroscopic digital camera system. Measurements were performed with Zeiss Axioplan Software, Version 4.1. Standard histological techniques were used to prepare sections of the leeches. Results of the analysis revealed that 61 of 186 (32.8 %) blood smears were positive, with the majority of infected crocodiles (34.5 %) presenting an infection prevalence of 1 out of 10 000 erythrocytes infected. Two crocodiles (3.2 %) had a very heavy parasite load of more than 1 out of 100 erythrocytes being infected. No significant difference was observed between the haematocrit values of crocodiles with or without a *H. pettiti* infection. Eleven of the 17 crocodiles (64.7 %) infested with leeches were positive for *H. pettiti*.

Micromorphology of the *Dinogamasus* mite and abdominal pouch of the host carpenter bee *Xylocopa caffra*

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Dinogamasus mites have only been found on some of the Old

World carpenter bees of the genus *Xylocopa* and in their nests tunnelled into old wood. These mites have no apparent negative or advantageous effect on the bees and yet the females of many species of *Xylocopa* have a specialised abdominal pouch in the 1st segment of the abdomen in which a number of these mites live. Four carpenter bees collected from a dwelling in Pretoria and fixed in 70 % ethanol were identified as *Xylocopa caffra*. The 1st abdominal segment was removed from each bee and the abdominal pouch carefully dissected to reveal the mites which numbered between 3 and 6. Both the mites and the pouches were routinely prepared for scanning electron microscopy and studied under a Leica stereoscan 420 at 5 to 10 kV. The *Dinogamasus* mites were all female and very large (approximately 1.7 mm in length), and characterised by a large dorsal shield with a species-specific pattern of setae. The legs were robust, with legs I and II bearing flattened conical setae which, along with certain setae on femur I, are of taxonomic importance. The microstructure of various setae and the ambulacral apparatus with its 2 claws and lateral spines gave some indication of how these mites may attach to and move on the bees. Other structures elucidated in this SEM study included the sternal, genital and anal plates as well as the pair of lateral spiracles with rudimentary peritremes near coxa III. Observations on the mouthparts confirmed that the fixed digit of the mandible was much shorter than the larger movable digit, which indicates that these are not parasitic mites. The abdominal pouch was an internal oval chamber opening onto the anterior surface of the abdomen by a single triangular entrance through which the mites must pass. This entrance was covered by a fringe of hairs for protection. Posteriorly the pouch is isolated from the abdominal viscera by a thin convex wall. The inner surface of the pouch was not smooth, but formed by overlapping lamellae which may improve traction for the mites.

Micromorphological specialisations of the sucking louse *Linognathus euchore* from the springbok, *Antidorcas marsupialis*

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Linognathus euchore is an obligate bloodsucking parasite of springbok which may cause anaemia during heavy infestation. The 5 species of *Linognathus* which have been described from springbok to date are morphologically very similar and thus difficult to identify, particularly the males due to their sexual dimorphism and a lack of diagrammatic descriptions in the literature. In this study the functional micromorphology of both sexes of *L. euchore* was investigated by scanning electron microscopy (SEM) as well as additional features of taxonomic importance. The lice collected live from springbok that had been brought to the state veterinary laboratories at Bloemfontein for *post mortem*, were fixed in 70 % ethanol. The lice were cleaned by ultrasonication before being routinely processed for SEM and viewed in a Leica Stereoscan 420 at 5 to 10 kV. The elongated conical head tapered from the enlarged ocular prominences to the haustellum at the anterior tip. Three pairs of hooked stylets for piercing the skin were observed protruding from the cone-shaped haustellum. The 2 large pore organs each contained a tuft organ and together with their associated plate organ, were borne on antennal segments IV and V. The thorax was deeply cleaved and bore a pair of long setae and a pair of large lateral spiracles. Legs I each bore a slender claw while the claw of II and III were extremely robust with well-developed scaled tibial pads for attachment. Abdominal segments III to IV bore single long marginal setae, while VI to VII were characterised by pairs of long marginal setae. The female gonopods IX were curved medially, each bearing a terminal tuft of setae, while gonopods X had a medial fringe of short setae. The abdomen of the male had a very large genital plate that reached from segment VII posteriorly and terminated in a single bulbous process. Each paramere had a characteristic longitudinal keel-like ridge. The everted male genitalia were also described for the 1st time.

Tongue replacement in marine fishes, a parasitic enigma

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The tongue replacement isopod is an uncommon, but most intriguing parasite of fishes, which is thought to have evolved from a pathogenic lifestyle to one exhibiting commensalism. This involves the movement from the external surfaces of the host, to the gills, the buccal cavity, and now the mouth. It is the only known occurrence where a parasite actually replaces a functional body part of the host. Over the past 3 years, various collection trips were undertaken to investigate the presence of tongue replacement among fishes from varying localities on the southern coast of South Africa. Pelagic and demersal fishes were captured using a German 180ft bottom trawl as part of the South African hake biomass survey. Infratidal and intertidal fishes were collected by hook-and-line and hand-held nets, respectively. Isopods found were fixed in 70 % ethanol and studied using light and scanning electron microscopy following standard techniques. Of the 18 different species of fishes screened, tongue-replacement isopods were found inhabiting 3, namely *Spondylisoma emarginatum* (steenjie), *Diplodus sargus capensis* (blacktail), and *Sparadon durbanensis* (musselcracker). The 2 inshore species (blacktail and musselcracker) were parasitised by *Ceratothoa imbricata* whereas the offshore species (steenjie) was parasitised by a *Ceratothoa* sp., unknown to science. This paper reports on new host, locality and species records for these tongue-replacement isopods in South Africa. It also confirms preference of tongue-replacement isopods to fishes of the family Sparidae.

Drug-resistant malaria in South Africa

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Malaria treatment failures in KwaZulu-Natal necessitated a change in treatment from chloroquine to sulphadoxine/pyrimethamine (SP) in 1988 and to an artemisinin combination during 2001. Similar treatment failures at a lower level were experienced later in Mpumalanga and Limpopo. Events during the appearance of drug resistance, assessment of resistance and implications of different treatment options to malaria control operations are described.

Cloning, expression and kinase activity of *Plasmodium falciparum* cyclin G-associated kinase

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Drug resistant cerebral malaria caused by *Plasmodium falciparum* remains a major health issue in Africa. The *Plasmodium* genome project provides the facility to search for novel drug targets. Recently, kinases have become attractive drug targets, and inhibitors have been introduced to treat cancer. Kinases are important in signal transduction processes and the control of the cell cycle. The objective was to identify and evaluate unique *Plasmodium* kinases as parasite selective drug targets. A putative homologue of cyclin G-associated kinase (*PfcGAK*) was identified. The catalytic domain and flanking region of *PfcGAK* was amplified by PCR and the sequence confirmed prior to directional cloning into an expression vector (pHB6) that yielded a fusion protein with a N-terminal HA- and a C-terminal His(6)-tag. Recombinant *PfcGAK* was isolated from induced cultures using Ni²⁺-affinity chromatography. Western blot analysis with anti-HA antibody and anti-His antibody detected a band of the expected size (65 kD). Screening of several clones also revealed the presence of a smaller than expected protein that contained both tags. Plasmid sequence analysis showed that a nanomeric repeat region of 81 amino acids was missing from the plasmid yielding the smaller protein. Recombinant *PfcGAK* of both sizes were used in kinase assays that showed that casein was the preferred substrate and that *PfcGAK* undergoes autophospho-

rylation. Both the 65 kD and 57 kD recombinant protein displayed kinase activity, indicating that the repeat region is not required for catalytic activity. In a preliminary screen with the kinase inhibitor roscovitine the autophosphorylation of PfcGAK was not inhibited. Further studies on the *in vivo* function of PfcGAK and on its kinase activity is currently under way. More data are required before a final evaluation of PfcGAK as drug target can be done.

Morphology of the anuran parasite *Eupolystoma vanasi* (Monogenea: Polystomatidae)

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Polystomatid flatworms are primarily parasitic in anuran hosts and to date there are 19 different genera. Of these, *Polystoma*, *Metapolystoma*, *Protopolystoma* and *Eupolystoma* are known to occur in South Africa, while the genus *Polystoma* has a cosmopolitan distribution, *Metapolystoma* and *Protopolystoma* are only known from Africa and *Eupolystoma* is restricted to Africa and India. A recently described species, *Eupolystoma vanasi*, occurs in the urinary bladder of the red toad, *Schismaderma carens*. Very little is known about this parasite and for the genus in general. The aim of this study was to study its morphology. The material for this study was collected from *S. carens* that were collected at Bela Bela in the Limpopo Province. Specimens were studied using ordinary light, scanning electron and confocal microscopy. In understanding the morphology of this parasite, a better understanding of the genus as a whole should be developed.

Okavango Delta – A haven for digenean parasites

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A digenean life cycle, as the name indicates, involves 2 or more hosts within its life cycle, snails usually being 1st intermediate hosts and fish 2nd intermediate or final hosts with piscivorous birds most likely acting as final hosts in many cases. During parasitological surveys from 1999 to 2003 in the Okavango River and Delta, snails and fish were collected and examined for the presence of larval and adult digeneans, for the purpose of elucidating different life cycles occurring in the Delta. A total of 8 different cercariae representative of 7 digenean families were shed from 6 different snail species, respectively. Current studies reveal an astounding 13 adult digeneans from 5 different fish species and a number of metacercarial forms from other fish species. One of these adults belongs to the same family as a cercarial form, the others belonging to totally different families. So out of 13 different life cycles, 1 of these has been elucidated. The questions therefore arise, are there more snail hosts shedding other cercariae within the delta, or is it possible that those snails already identified actually shed more cercariae than we have found so far? Further investigations should focus on potential seasonality in cercarial shedding; seasonality in snail occurrence, with snail aestivation; fluctuating river levels; migration of bird species as well as fish migration and seasonal behaviour in the system.

Psychiatric manifestations of parasitic infections

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Neurocysticercosis is the most common parasitic infection of the human central nervous system. Clinical signs vary depending on the size and stage of the parasites and the anatomical location in the

brain. Various psychiatric symptoms are known to be caused by this infection. Focal seizures and hydrocephalus with neuropsychiatric symptoms are common presentations, but organic mental disorders with visual, olfactory and auditory hallucinations may occur. Depression rather than psychosis seems to be the most common symptoms. Toxoplasmosis is a typically opportunistic infection that causes focal lesions in the brain and that may accompany HIV Aids infection. Various psychotic symptoms, as well as seizures and other neurological findings, may result from cerebral toxoplasmosis. Of the systemic infections, malaria may cause a diversity of psychiatric effects, including delirium and coma, malaria psychosis and depression. Trypanosomiasis is frequently associated with psychiatric symptoms especially mania and depression. In Gambian trypanosomiasis confusion, hallucinations and antisocial behaviour are common. Depression may be an important feature of Chagas disease, the causative agent which is related to that of trypanosomiasis. Unusual psychotic symptoms and aggressive behaviour have been reported in children with amoebiasis and giardiasis. Infections with *Loa loa*, *W. bancrofti* and *Onchocerca volvulus* may present with a range of psychiatric symptoms which may be either acute or chronic. The chronic illness fails to remit after leaving the tropics. These symptoms include personality change and impaired intellectual functioning. Any psychiatric symptoms like disturbance of consciousness, mood, anxiety, psychosis and personality changes should be detected/evaluated when cerebral parasitosis is suspected and the appropriate special investigations should be done to confirm the diagnosis.

The role of parasitic wasps in integrated tick management

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The conventional method of tick control using chemical acaricides is fraught with various problems such as environmental pollution, tick resistance and the exorbitant cost of acaricides. Alternative methods of tick control for alleviating these problems are therefore needed. Parasitic wasps (parasitoids) have produced excellent results in the biocontrol of plant pests. Although 7 parasitoid species of ticks belonging to the genus *Ixodiphagus* are reported in the literature, only *Ixodiphagus hookeri* and *I. texanus* have been studied in-depth under laboratory conditions. For unknown reasons, trials to introduce *I. hookeri* to a number of sites have either failed or produced little impact on tick populations. In a survey of tick parasitoids conducted in Kenya, a tick parasitoid was found infesting nymphs of *Amblyomma variegatum* at various localities at very different degrees of prevalence, ranging from 0 % to 70 %. The parasitoid was unable to parasitise the other life stages of *A. variegatum* and all stages of other tick species suggesting that it was specific for *A. variegatum* nymphs. Release of this parasitoid near cattle infested with ticks drastically reduced on-host cattle populations of *A. variegatum*, but did not affect other tick species on the same hosts. Release of this parasitoid in a locality where it did not naturally occur failed to establish infection in *A. variegatum* infesting cattle. This suggests that environmental factors influence the occurrence and infectivity of the parasitoid. The positive results obtained from the pilot field release, however, suggest that parasitoids can be candidates for integrated control of ticks provided that the packages are carefully constituted and exclude chemical acaricides. Further detailed studies on the biology and ecology of tick parasitoids as well as their interaction with other tick control methods are, however, needed before they can be incorporated into tick control strategies.

Malaria vector situation in the Limpopo Province

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The different species of malaria vector mosquitoes found in the Limpopo province are discussed in order to put in context their roles in the transmission of the disease. The 2 main vector groups of the *Anopheles gambiae* and the *Anopheles funestus* groups are responsible for transmission in South Africa. An historic account of their roles

in transmission over the past 50 years is also discussed. For the disease to thrive there needs to be the parasite and the vector. Since the control programmes focus mainly on controlling the vector, it is only logical that effort be put into studying the vectors in this province. The MCP has over the years put many resources into studying their biology and tracking the vector populations in the province. Variables in the vector biology, *i.e.* such as resting and feeding behaviour, resistance to insecticides, have had a major impact on the way the control programme has been run over the years. A few experiences will be presented.

Presence of a cestode larva in the uterus of another cestode species

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Specimens of *Clarias gariepinus* were collected from Loskop Dam using gill nets and transported alive to a field laboratory where they were killed and examined for parasites. Cestodes were killed and fixed in alcohol-formal-acetate (AFA) and preserved in 70 % alcohol. Whole mounts were stained in Alum Carmine and sections in Azan. They were dehydrated in a series of alcohols, cleared in xylene and mounted in Entellan.

A very large, previously unknown mature cestode was collected from the intestine of *C. gariepinus*. To study the anatomy of this cestode, longitudinal sections (5 mm thick) of the proglottids were made. While studying these sections, a larval cestode was found in the uterus of 1 mature proglottid. The scolex of the adult cestode differs markedly from the scolex of the larva. It has 4 acetabular suckers and does not have a rostellum or hooks; while that of the larva has 4 acetabular suckers and a retractile rostellum with hooks. The adult cestode belongs to the Order Proteocephalida, since it has a scolex with 4 acetabular suckers and follicular vitellaria. It has many similar characteristics with members of the subfamily Proteocephalinae, but differs from the latter group in that the ovary and uterus are partly cortical and partly medullary. In this respect, it resembles members of the family Monticelliidae but differs from the latter group in that the vitellaria are medullary and not cortical as in the family Monticelliidae. The larval cestode either belongs to the Order Cyclophyllida or subfamily Gangesiinae (Proteocephalidae) but differs from the latter group in that its rostellum is retractile. The reproductive organs of the larval cestode were not yet developed and could not be compared with those of the adult cestode.

A preliminary assessment of mitochondrial DNA diversity among specimens of *Nesippus orientalis* (Siphonostomatoida, Pandaridae) from a variety of shark hosts off the east coast of South Africa

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Nesippus orientalis Heller, 1868, is a cosmopolitan copepod parasite of a wide variety of elasmobranchs. It is found mostly on the gill arches and inside the mouth of their hosts. Morphologically it belongs to the *Dinemoura*-group of the Pandaridae, which is distinguished from the *Pandarus*-group by the absence of dorsal plates on the 2nd free thoracic segment. Specimens of *N. orientalis* are distinguished from the other 3 nominal species of *Nesippus* by the presence of dorsal plates on the 4th thoracic segment. The main objective of this preliminary molecular study was to determine mitochondrial DNA Cytochrome Oxidase I diversity among *Nesippus orientalis* sampled from a variety of spatial scales along the KwaZulu-Natal coast. We used standard methods of DNA extraction, PCR and automated DNA sequencing. The DNA sequences were analysed phylogenetically using PAUP and TCS. The results

show a strong phylogenetic signal between parasites from *Sphyrna mokarran* and *Carcharhinus limbatus* versus the remainder of the host species. Within the large parasite clade (from *Carcharias taurus*, *Sphyrna zygaena*, *Carcharodon carcharias* and *Isurus oxyrinchus*) there was no link between genetic relationships and specimen/parasite locality. Future research will include more shark species and an intensive study of parasites from *Carcharias taurus* from a broad geographical area.

Utilisation of host specificity of monogeneans as a taxonomic indicator of host yellowfish species

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Recent genetic studies have shown that it has become increasingly difficult to distinguish between *Labeobarbus aeneus* and *L. kimberleyensis* on a mitochondrial DNA level. These yellowfish species are hosts for the monogenean parasite *Paradiplazoon* species. Thus, the possibility of using the monogenea of endemic *Labeobarbus* species, incorporating allozyme studies and morphometrics as a taxonomic indicator is raised. Published data clearly showed a high degree of host specificity in marine monogeneans. Specimens collected from these studies indicated that 78 % of the 425 monogenean species are restricted to 1 host species, 89 % to 1 genus, 96 % to 1 family and 98 % to 1 order. Morphometric measurements and allozyme analyses were done on *Labeobarbus polylepis* from the Elands River, as well as *L. aeneus* and *L. kimberleyensis* specimens from the Vaal River systems. *Paradiplazoon* parasites were studied from *L. aeneus* and *L. kimberleyensis*. The genetic data were analysed with Biosys-2 software and results amongst the different fish species were compared. Goodness of fit statistics showed a consistent grouping of *L. polylepis* and *L. aeneus* with possible hybrid fish more closely related to this clade. *Labeobarbus kimberleyensis* occurred as a sister group to the above-mentioned samples. No fixed allozyme markers were found for the different fish species in this study. This indicates introgressive hybridisation between the yellowfish species studied. *Paradiplazoon* specimens from *L. aeneus* and *L. kimberleyensis* were stained using Horen's Trichrome solution and morphometric data were recorded for statistical analysis and compared with the fish morphometric and genetic results. The attachment clamps of the parasite specimens reacted positively for autofluorescence. A 2nd group of *Paradiplazoon* specimens from the same host species were stained using a cell contamination probe kit to specifically stain alpha-acetyl-glucosamine, which constitutes the chitinous attachment organs. This finding corroborated that the clamp structure could be a taxonomic indicator for *Paradiplazoon* sp. The *Paradiplazoon* sp. showed a stenoxenic specificity as expressed by the fact that the same species occurs only on fish in the subgenus *Labeobarbus* whereas the *Labeo* species in the Vaal Dam host a different parasite species.

Monogenean parasites on gills of freshwater fish from the Okavango Delta, Botswana

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Monogeneans are ectoparasites of both marine and freshwater fish where they are found on the gills and skin, and are known to be highly host- and site-specific. These parasites have a unique attachment organ (opisthaptor) at the posterior end of the body which destroys epithelial tissue around the attachment site. The objectives of this project were to identify, study the morphology, and taxonomically classify these parasites. Field collections of different freshwater fish species were made from different localities in the Okavango Delta, Botswana. Fish species were identified and examined for gill monogenean parasites which were then studied by light and scanning electron microscopy using standard techniques.

Monogeneans from the following fish species are reported on: *Cichlidogyrus halli*, *C. dossoui*, *C. sclerosus*, *C. quaestio* and *C. bouvii* from *Tilapia rendalli*; *C. zambezensis* from *Serranochromis robustus*; and *S. altus*, as well as *Schilbetrematoides manzini*, from *Schilbe intermedius*; and an undescribed species from *Hydrocynus vittatus* and *Sargochromis carlottae*. These fishes were infected with different monogenean parasites, each with its unique opisthaptor that bears different combinations of clamps, bars and marginal hooklets. Therefore, it is very important to study monogenean genetics in order to gather more information on how monogenean genera restrict their host range to fish of 1 family and/or alternatively only a few related fish genera within a family.

Description of digenean trematode larval stages belonging to the family Strigeidae in proximity of Pretoria, Gauteng

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The family Strigeidae is a major group in the superfamily Strigeoidea/Diplostomatoidea that includes the genus *Strigea*, of which the adults parasitise in the intestines of piscivorous birds. The life cycle usually consists of freshwater snails serving as 1st intermediate hosts, fish as 2nd intermediate hosts and birds as final hosts. This research project concentrated mainly on the descriptions of the larval stages in snails and fish belonging to the family Strigeidae. Freshwater snails were collected around Pretoria whereafter their cercarial sheddings were studied by using light and scanning electron microscopy techniques. Fish, freshwater shrimps and other invertebrates were also collected and examined for metacercariae, which were studied with the aid of light microscopy. Three different types of snails were collected from different waterbodies which secreted a total of 4 different types of strigeid cercariae, namely *Lymnaea natalensis* that secreted 2 types, as well as *Bulinus tropicus* and *Ferrissia fontinalis* that both secreted only 1 type. Three types of strigeid metacercariae were also collected from muscle tissue: 2 from *Pseudocrenilabrus philander* and 1 from *Clarias gariepinus*. The results of study lays a foundation for the continuation of research on these digenetic trematodes because their life cycles are unknown and should be investigated experimentally. The study also provided insight into the different types of digenetic parasites belonging to the family Strigeidae that are present in waterbodies in the proximity of Pretoria.

Indications of endemically stable vector-borne protozoal infections in African wildlife

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Bovine babesiosis serves as a model for studies on endemic stability. Virtually every wild ungulate and carnivore species seems to harbour at least some haemoprotozoan parasites. High prevalence rates have been recorded. Indications are that endemically stable conditions also exist in free-ranging wildlife populations. Recent advances in diagnostic techniques, e.g. molecular characterisation, have opened a new window of opportunity for studying vector-borne protozoal infections in free-ranging wild animals. In general, the situation fits the bovine babesiosis model, i.e. absence of clinical cases where the parasites are prevalent. There are various scenarios under which clinical disease will manifest. One is where animals grow up in the absence of challenge (e.g. zoo-bred) and are exposed to the parasites at a later stage (introductions of ungulates often occur indiscriminately). Another is where immune animals are stressed, and latent infections flare up, e.g. *Babesia leo* in lions, *Babesia bicornis* in black rhinos and *Theileria equi* in Grevy's zebras. Veterinarians and wildlife managers should be aware of the situation, and prophylactic administration of drugs may be called for.

The impact of 2 dipping systems on endemic stability to bovine babesiosis and anaplasmosis in cattle in 4 communally grazed areas in the Limpopo Province, South Africa

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A 12-month study was conducted in 4 communal grazing areas in the Bushbuckridge region, Limpopo Province, South Africa. The main objective was to investigate the impact of reduced acaricide application on endemic stability to bovine babesiosis (*Babesia bigemina* and *Babesia bovis*) and anaplasmosis (*Anaplasma marginale*) in the local cattle population. To this end 60 cattle at each communal grazing area were bled at the beginning and the conclusion of the experimental period and their sera were assayed for *B. bovis*, *B. bigemina* and *Anaplasma* antibodies. Cattle in the intensively dipped group were dipped 26 times and maintained on a 14-day dipping interval throughout the study, whereas cattle in the strategically dipped group were dipped only 13 times. Three cattle, from which adult ticks were collected, were selected from each village, while immature ticks were collected by drag-sampling the surrounding vegetation. At each diptank a questionnaire aimed at assessing the prevalence of clinical cases of tick-borne disease, abscesses and mortalities was completed by an Animal Health Technician, during the dipping process. An increase in seroprevalence to *B. bovis* and *B. bigemina* and a decrease in seroprevalence to *Anaplasma* was detected in the strategically dipped group, whereas in the intensively dipped group the converse was true. *Amblyomma hebraeum* was the most numerous tick species on the cattle, and *Rhipicephalus (Boophilus) microplus* was more plentiful than *Rhipicephalus (Boophilus) decoloratus*. Drag samples yielded more immature stages of *A. hebraeum* than of *Rhipicephalus (Boophilus)* spp. The incidence of clinical cases of tick-borne disease and of abscesses increased in the strategically dipped group at the start of the survey.

Characterisation of South African *Theileria parva* isolates

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Differentiation between *Theileria parva* subtypes has traditionally been based on differences in the numbers of schizonts and piroplasms present in infected animals and the epidemiology of the disease. In this study, Restriction Fragment Length Polymorphism (RFLP) analysis of the variable regions of the parasite antigen genes *p104*, *PIM* and *p67* was used to discriminate between *T. parva* isolates. Parasite DNA was extracted from blood collected from buffaloes from Hluhluwe, Mabalalingwe, Marekele, Free State and Kruger National Park and cattle from KwaZulu-Natal (Mr Green's farm) and Welgevonden. *p104*, *PIM* and *p67* genes were amplified and analysed. In East Africa a 130 bp insert in the *p67* gene has been used to distinguish buffalo-derived from cattle-derived *T. parva* isolates. Most of the buffalo-derived isolates in this study lacked the insert, indicating that this criterion cannot be used to distinguish between buffalo- and cattle-derived isolates in South Africa. This finding does not rule out the possible existence of other markers in the *p67* gene. The majority of the *p104* RFLP profiles were typical of buffalo-derived isolates. A new profile was identified from the Welgevonden isolate, while the profiles of isolates from cattle on Mr Green's farm were similar to that of Muguga, a cattle-derived isolate from Kenya. *PIM* profiles from these isolates were homogenous and also resembled Muguga. Most of the buffalo-derived isolates had highly polymorphic *PIM* profiles, except for those from the Hluhluwe buffalo which were homogenous. The *PIM* profile of the Free State isolate was similar to the Hluhluwe profile. The *p67*, *p104* and *PIM* profiles can be used to distinguish between *T. parva* isolates, but we do not yet know if the profiles correlate with the pathology of the isolates.

Investigation of the gastrointestinal tract of larval and juvenile *Argulus japonicus* Thiele, 1900

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Previously the gastrointestinal tracts of adult branchiurans (*Argulus*, *Chonopeltis* and *Dolops*) have been studied and it was found that they are similar in structure and function. However, little knowledge is available on the gastrointestinal tract of larvae, the literature on this subject is scant and contradictory and was based on superficial observation. Previous researchers for instance observed that the 1st larval stage of *A. foliaceus* contains a large amount of yolk in its gut. It was therefore speculated that the very early larval stages do not need a host on which to feed. This seems to concur with the opinion of other researchers who claimed that newly hatched *A. foliaceus* larvae swim 2–3 days seemingly without the need of a host, i.e. nourishment. Others claimed that they fed on host tissue once they have hatched in their 1st stage and observed that the newly hatched larvae of *A. coregoni* were parasitising the fish, *Rhodeus ocellatus*, as well as that larval *A. foliaceus* congregated around the areas with most mucus on their hosts, perhaps suggesting that feeding took place. The stage at which argulid larvae begin feeding on fish tissue was therefore difficult to determine. *Argulus japonicus* was introduced into South Africa and occurs in large numbers (up to 1300 per fish) on indigenous fish species. The adult parasites feed on blood and a heavy infestation leads to the death of hosts. Owing to the large numbers that occur in South Africa, *A. japonicus* larvae were chosen for this study. The gastrointestinal tracts were sectioned and reconstructed; the cells of the gut were also studied and compared to that of the adult. The cells that line the midgut of the newly hatched larva harbour large vacuoles containing yolk. It was observed that larvae must find a host within 1 day in order to survive, and the parasite begins feeding on the cells and mucus of the host already in the 1st larval stage. The larvae, however, are not able to feed on fish blood as their mouthparts are not long enough to penetrate fish skin. It is therefore concluded that *Argulus japonicus* is parasitic its entire lifecycle and there are no free-living stages.

Malaria in pregnancy in KwaZulu-Natal

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Malaria in pregnancy is associated with adverse effects such as anaemia, low birth weight, prematurity, stillbirth, miscarriage and maternal death. To prevent these effects, the World Health Organization recommends implementation of Intermittent Preventive Treatment (IPT) for all pregnant women at risk of malaria. In South Africa, information is required to support implementation of IPT policy. A study was undertaken during 2004/2005 to determine the burden of malaria in pregnancy and the effects of malaria on birth outcomes in KwaZulu-Natal. Data were collected from pregnant women attending antenatal care clinics and included demographic details, routine antenatal care attendance, current and previous malaria infections during pregnancy, use of insecticide-treated bed nets and birth outcomes. The 1101 pregnant women studied were characterised by high levels of teenage pregnancy, functional illiteracy, unemployment and being single. A fifth of these women mentioned that they owned a bed net and more than a third said that they were using these bed nets. All 409 women tested for malaria were negative. Based on self-reported history, 1.9 % of women had been infected with malaria during pregnancy. The levels of anaemia and HIV in pregnant women are very high in comparison to malaria infection. To conclude, the burden of malaria in pregnant women is very low and this suggests that pregnant women may have benefited from aggressive malaria control in this area. Therefore, the prevalence of anaemia and HIV cannot be associated with malaria. These findings provide no basis for recommending IPT for prevention of malaria in pregnancy in KwaZulu-Natal.

Aspects of the digestive system of *Lamproglena clariae* (Copepoda: Lernaecidae) collected from the gill filaments of *Clarias gariepinus*

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Adult female specimens of *Lamproglena clariae* attach to gill filaments of *Clarias gariepinus* and consume blood. The current study was aimed at describing the digestive tract of this parasite. Specimens of *L. clariae* were collected from the gill filaments of *C. gariepinus*, fixed, prepared for histological examination and stained with Heidenhein's azan solution. A simple digestive system with 3 portions, the foregut, the midgut and the hindgut were observed. The foregut consists of a highly muscularised, cuticularised oesophagus lined with epithelium. The thick layer of muscles allows the oesophagus to stretch and contract while ingesting host tissue. The oesophagus extends through a funnel structure into the anterior midgut. The midgut is divided into 3 zones with different types of cells. The anterior zone of the midgut is lined with a thin layer of simple cuboidal epithelium followed by the median zone, lined with columnar epithelium layer. These 2 anterior zones of the midgut are involved with the digestion of ingested host tissue. The posterior zone has a thin layer of vacuolarised cuboidal epithelium and is involved in absorption of digested material. The hindgut is very short, cuticularised and has muscles probably involved in defecation and intake of water by reverse peristalsis. These observations show that the morphology of the digestive system of *L. clariae* is suitable for its sucking mode of feeding.

Antimalarial activity of new 1-*N*-substituted cyclised pyrazoline analogues of thiosemicarbazones

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Malaria remains a major reason for morbidity and mortality in Africa and India. The malaria parasite has developed resistance to the antimalarials currently being used; thus, new effective agents are being synthesised. The thiosemicarbazone class of compounds has been reported to possess a wide spectrum of antimicrobial activity against mycobacteria, bacteria, trypanosomes and protozoa. Based on this, new cyclised pyrazoline analogues of thiosemicarbazone were synthesised with the thiocarboxamide moiety being substituted by aliphatic, cyclic and aryl amines. The chloroquine-resistant *Plasmodium falciparum* malaria parasite was cultured *in vitro* and the sensitivity of the parasite to the thiosemicarbazone derivatives was evaluated using the tritiated hypoxanthine incorporation assay. Out of 24 compounds evaluated for antimalarial activity, the compounds with a 2-chloro benzyl amine (S2) and *n*-phenyl piperazine (S5) as a 1-*N* substitution and bromine at position 3 of the phenyl ring were found most active with IC₅₀ values of 17.14 ± 1.88 M and 18.98 ± 1.59 μM, respectively. It was concluded that a 3-bromo substituent on the phenyl ring at position 3 of the pyrazoline ring and bulkier groups at thiocarboxamide enhanced the antimalarial activity. S2 interacted in an additive manner with both chloroquine and quinine. Thus, to improve the activity of these preliminary compounds, further chemical modifications will be performed.

Confocal microscopy examination of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*

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The amphibian chytrid fungus *Batrachochytrium dendrobatidis* causes cutaneous chytridiomycosis, a disease responsible for amphibian population declines around the globe. The morphology of *B. dendrobatidis* has been well documented through conventional and cryo scanning electron microscopy and transmission electron

microscopy. The present study is not a redescription of the organism, but rather a review of the molecular characteristics pertaining to morphology and development as revealed by laser scanning confocal microscopy (LSCM). Various tissue types were prepared for LSCM including haematoxylin and eosin-stained histological sections of amphibian skin, fixed and fresh skin samples as well as live *B. dendrobatidis* cultures. Samples were labelled with 4 different dyes: Acridine Orange (nucleic acid probe), Nile Red (lipid probe), Tetramethylrhodamine methyl ester (TMRM, membrane potential probe) and LysoTracker (acidotropic probe). All dyes were applied for 15 min. The surface and septa of sporangia react strongly with TMRM and Acridine Orange. Nile Red effectively illustrated the configuration of the lipid bilayer of the cell membrane and the lipid globules of zoospores. LysoTracker specifically bound to acidic organelles in the sporangium, zoospores and rhizoids. It is not recommended that any of the tested dyes be used for diagnostic purposes of *B. dendrobatidis*, but rather as probes that can associate with unique fungal characteristics such as chitin and glucan found in the cell wall should be used. LSCM could be very useful when attempting to answer specific questions regarding the biology and pathology of *B. dendrobatidis* that are not yet fully understood.

Biodiversity and community structure of the parasites of 2 endemic catshark species from the west and south coasts of South Africa

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The current study focuses on 2 endemic catshark species: the dark shyshark, *Haploblepharus pictus*, and the puffadder shyshark, *H. edwardsii*. These small sharks, reaching a length of 60 cm, are found from Namibia to Agulhas (*H. pictus*), and Cape Point to northern Natal (*H. edwardsii*). Sharks were collected by scuba divers from 6 sites from Lambert's Bay to De Hoop Nature Reserve. Parasites from the skin, gills, body cavity, stomach and intestine were counted, removed, and fixed as appropriate. A total of 143 sharks was examined. The sharks were found to host representatives of a large variety of different parasitic taxa, *i.e.* Crustacea, Trematoda, Nematoda, Hirudinea, Cestoda and Protozoa, including a number of species new to science. Multivariate analysis (Primer software) was used to detect differences in parasite assemblage, a) between the 2 host species; b) on each host species among different geographical areas; and c) among size classes of each host species; all of which were found to be significant, with implications for the application of studies on parasite assemblages in marine ecology. There is a notable paucity of research into marine parasite assemblages.

Poster Presentations

18S sequence data reveal genetic diversity in *Theileria equi* and *Babesia caballi*

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Genetic diversity amongst the protozoal parasites that cause equine piroplasmiasis was revealed during development of a real-time PCR assay. Simple probes based upon sequence differences in *B. caballi* and *T. equi* 18S rRNA genes were designed and used to detect and distinguish between *B. caballi* and *T. equi* infections using a Lightcycler. Samples that tested positive in culture for either *B. caballi* or *T. equi* were used as positive controls in the development of the real-time PCR assay. We did not obtain positive signals from all of the samples. Therefore the reverse line blot assay was used to screen 120 samples known to be positive for either *T. equi* or *B. caballi*. Samples that hybridised to the *Theileria/Babesia* catch all probe only

were selected for further analysis. Primers designed in conserved domains of the 18S rRNA gene of *Theileria* and *Babesia* species were used to amplify and sequence the hypervariable V4 region for each sample. Sequences were assembled using the Staden software and multiple sequence alignments were performed using ClustalX. Sequence analysis revealed variation in the 18S sequence, which explained the failure of the real-time PCR assay to detect all samples.

Phylogenetic analysis of *Babesia* parasites in cheetahs in South Africa

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The Reverse Line Blot hybridisation assay (RLB) was used to screen 300 blood samples collected from lions, cheetahs, a black-footed cat, a serval and domestic cats. The assay is based on the amplification of a 460 to 520 bp fragment in the V4 variable region of the 18S rRNA of *Theileria* and *Babesia* species, and the subsequent hybridisation of the PCR product to *Theileria* and *Babesia* species-specific probes. *Babesia* and/or *Theileria* genus-specific probes, as well as *B. felis*, *B. leo* and *Cytauxzoon felis* species-specific probes were included. Although some of the cheetah blood samples tested positive for *B. felis* and *B. leo*, a large number (29.45 %) gave a positive signal for only the *Babesia* and/or the *Theileria* genus-specific probe. The PCR products of these samples were further analysed by sequencing. Sequencing data were analysed and edited using the Staden package, and aligned with published sequences of related genera by using the ClustalX program. Phylogenetic trees were constructed using neighbor-joining in combination with the bootstrap method. The sequence similarity analysis showed that isolates obtained from the cheetah blood grouped closely with *B. gibsoni*, previously described in dogs and a *Babesia* parasite isolated from caracal. These results support the possibility of the presence of unknown *Babesia* species in cheetahs in South Africa.

Biodiversity of myxosporeans infecting intertidal fish in the Tsitsikamma National Park

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Myxosporeans are endoparasitic metazoan animals, with only a few species having been reported from South Africa and mostly those infecting economically important fishes, such as the Cape snoek. As part of a larger project on the biodiversity of the Tsitsikamma National Park coast, intertidal and infratidal fishes were collected using the bailing method, hand nets and hand lines. Ten *Clinus cottoides*, 10 *C. superciliosus*, a single *Amblyrhynchotes honckenii* and a single *Sparadon durbanensis* were collected and kept in an aerated tank until they were anaesthetised using clove oil and their spinal cords cut behind the head. The skin, gills, gall and urinary bladders were screened for the presence of myxosporean infections. Photographs were taken of any observed infection with the aid of a Nikon Coolpix digital camera mounted on a Nikon compound microscope. Samples from the gills were taken and prefixed in a solution of Acetic acid for 24 hours and subsequently post-fixed in Davidson's solution and prepared for histology following standard techniques. *Clinus cottoides* yielded *Myxobolus* infections in the gall bladders of 6 of the 10 fish. Six of the *C. cottoides* gall bladders were infected with a *Ceratomyxa* sp. One *C. cottoides* yielded a *Hennequya* infection and another *Myxobolus* sp. encysted on the gills. The gall bladders of all 10 *C. superciliosus* were infected by a *Ceratomyxa* sp. A *Myxobolus* infection of the gall bladder was also observed in *S. durbanensis*. No infections were observed in the *A. honckenii*. Pathology caused by the *Hennequya* and *Myxobolus* gill cysts was observed by histology. Cysts were found to be in the primary and secondary filaments, greatly reducing the surface area needed for oxygen diffusion. Some of the species of myxosporeans found in Tsitsikamma National Park have been reported from the same fish hosts at De Hoop Nature Reserve and Jeffrey's Bay.

For all these species this is a new locality record. The discovery of a *Myxobolus* sp. in the gill and gall bladder of *C. cottoides* and *S. durbanensis* is a new record for both this parasite and host, which may be a new species. Further research will have to be done, however, in order to confirm this.

In vitro antiplasmodial activity of cyanocobalamin

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Cyanocobalamin (vitamin B₁₂) is a complex molecule containing a cobalt atom in the centre of a corrin ring, which resembles the iron-containing porphyrin ring found in haemoglobin and cytochromes. Ferriprotoporphyin is known to be toxic to the malaria parasite and is polymerised to an inert haemozoin crystal within the parasitic food vacuole. It is therefore proposed that vitamin B₁₂ by virtue of its structure may interfere with the formation of the haemozoin crystals. The effects of vitamin B₁₂ and 4 of its derivatives, dicyanocobinamide, coenzyme B₁₂, aquocobalamin and methylcobalamin were tested on the *in vitro* growth of *Plasmodium falciparum* using the tritiated hypoxanthine incorporation method. The ferriprotoporphyin biomineralisation inhibition test was carried out under acidic conditions to mimic the process of haemozoin formation in the parasitic food vacuole. The effects of the vitamin B₁₂ derivatives were compared to the 8-aminoquinolines, chloroquine and quinine which are known to inhibit haemozoin formation. Coenzyme B₁₂ and aquocobalamin were the most active in inhibiting parasite growth with IC₅₀ values of 2.42 ± 0.37 μM and 8.94 ± 0.60 μM, respectively. All cobalamins except dicyanocobinamide were more potent inhibitors of β-haematin formation compared to the 8-aminoquinolines. Therefore, 1 mechanism by which the cobalamins inhibit malaria parasite growth is by interfering with the formation of haemozoin and may have a protective effect against this protozoal infection.

Characterisation of an intracellular protozoan parasite infecting the digestive gland of abalone, *Haliotis midae* in South Africa

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Abalone is a very important economically exported shellfish worldwide. Numerous parasites and diseases pose threats to the production of abalone. These factors have been extensively studied in the past. Recently a new parasite has been found in the digestive gland of *Haliotis midae* farmed in the Western Cape Province. It is to date an unidentified parasite possessing apicomplexan characteristics. Abalone samples were collected from 3 abalone farms, Abagold (Hermanus abalone) in Hermanus, I&J in Gansbaai and Jacobs Bay Sea Products in Jacobs Bay. At each farm 30 abalone were collected from the holding tanks and removed from their shells. The abalones were killed according to accepted methods, their digestive glands removed, and cut into 3 pieces. Samples were placed into histological cassettes and fixed in Davidson's fixative for 24 hours and post-fixed in 70 % ethanol. From every sample 1 piece of digestive gland was prepared for histological studies using standard methods. The remaining 2 sections were prepared for transmission electron microscopy, also following standard techniques. Preliminary results indicated that 10 % of the abalone examined were infected with this enigmatic apicomplexan parasite. This paper reports on the finer structures of this parasite and its possible taxonomic position.

A new species of gnathiid isopod parasitising 2 species of requiem sharks from Lizard Island, Great Barrier Reef, Australia

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Gnathiid isopods are one of the most common ectoparasites of coral reef fishes. Only the juvenile stages are parasitic. These engorge on elasmobranch and teleost blood before returning to the reef to digest their meal and to moult into their next life stage. Currently, only 2 species of gnathiids, *Gnathia pantherina* from South Africa and *Gnathia trimaculata* from Australia, have been described parasitising elasmobranchs. During April 2002 a 3rd species was found attached to the gill filaments and gill arches of black tip reef sharks, *Carcharinus amblyrhynchos*, and a white tip reef shark, *Triaenodon obesus*, caught at Lizard Island on the Great Barrier Reef, Australia. Since the taxonomy of gnathiids relies on the morphology of adult males, larvae were cleaned and kept in 50-ml specimen jars filled with fresh seawater until they moulted into adults. Larvae started to moult into males from 14 to 19 days post-feeding. Egg development in female larvae was observed from 19 days post-feeding. None of these female larvae completed their moult into adults. Descriptions are based on light and scanning electron microscopy observations of the male and larvae. Unique features of the male include the triangular inferior medio-frontal process, 2 distinguishable areolas on the dorsal side of the pylopod and a twice as long as wide slender pleotelson with concave lateral sides. This species is the largest of the known species infesting elasmobranchs.

Morphology of the male and immature specimens of *Nesippus orientalis* Heller, 1868 (Pandaridae: Siphonostomatoida)

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Nesippus is one of the 14 accepted genera of the Pandaridae and currently has 4 nominal species. *Nesippus orientalis*, a cosmopolitan species found in the mouth and on the gill arches of a number of sharks and is distinguished from the other species by the presence of dorsal thoracic plates on the 4th segment. Specimens were collected from various sharks caught in the nets of the Natal Sharks Board, KwaZulu-Natal, South Africa. Collected specimens were preserved in 70 % EtOH and studied using the wooden slide technique. Immature and young females were among the collected specimens as well as males, some of which were still holding on to the young females. The maxillipeds of the male have a slightly different structure than that of the female and are used in holding on to the female while presumably transferring spermatophores to the female. Apart from the maxillipeds the general morphology of the male closely resembles that of the young female. This is in accordance with Heller's differences between male and female in 1868, differences which were discarded as the differences between a young and adult female by Wilson in 1907. The only specimen found during this study that closely resembles Wilson's 1907 description of the male was an immature female.

Micromorphology of the larva of the greater waxmoth *Galleria mellonella*

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Galleria mellonella parasitises honey bee colonies where the larvae not only consume and destroy the honeycombs but the mature larvae also damage wooden hives by chewing hollows in the wooden frames in which they spin their cocoons. This study investigated the functional external micromorphology of the mature larvae by scanning electron microscopy (SEM). Mature larvae were collected from infested beehives near Port Alfred and fixed in 70 % ethanol. The larvae were cleaned by ultrasonication before being routinely processed for SEM and viewed in a Leica stereoscan 420 at 5 to 10 kV. The study of the head revealed specialisations for feeding, including palpi with sensilla for sensing the food, and the large robust mandibles with double medial notches for ingesting the honeycomb and chewing the wood. The oral cavity was surrounded

by a funnel-like membrane to collect the food. The integument of the larva was membranous, but covered with small scales with scalloped edges which interdigitated with the adjacent scales. A pair of oval spiracles was carried laterally on the head, the metathorax and abdominal segments I to VIII. The luminal surface of each was covered by a matt of branched processes which may function to filter the air entering the trachea. The thoracic segments were clearly divided with each bearing a pair of clawed legs. Additional pairs of sucker-like prolegs were borne on the abdominal segments III to VI and a large pair of posterior suckers on the terminal segment. The inner surface of each sucker was longitudinally folded for expansion while between 18 and 28 hooks lined the inner surface. These would make effective hold-on organs for the larvae. The posterior suckers were horseshoe-shaped surrounding the centre which contained elongated hooked processes for attachment on rough surfaces.

Presence of f2 antibodies during the course of a *Fasciola gigantica* infection in cattle

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Fasciolosis, or liver fluke, is a helminthosis caused by the trematodes, *Fasciola hepatica* and *F. gigantica*, which settle in the bile ducts of many different species. It is a disease of great economic impact and therefore the early diagnosis of the disease is important. Faecal egg counts are commonly used to diagnose an infection in livestock. However, shedding of eggs by adult flukes is irregular and an infection with the parasites can easily be missed. An ELISA developed by the Institut Pourquier in France was shown to be a very sensitive method for the diagnosis of fasciolosis in cattle. The objective of this study was to determine how soon infected animals will become sero-positive and how soon after they have been treated for fasciolosis, will they become sero-negative. Four sero-negative animals were artificially infected with approximately 400 *Fasciola gigantica* metacercariae each. After infection was confirmed with faecal egg counts the animals were treated with products registered for the treatment of *Fasciola* spp. (active ingredient is triclabendazole). During the study blood samples were collected from all the animals on a weekly basis. The serum was used to test for antibodies against the f2 antigen. The results showed that the immune response can be detected between 7 and 14 days after infection and that the animals will become sero-negative approximately 12 weeks after they were treated. The ELISA is therefore an effective method to diagnose early fasciolosis in cattle, but can give positive results for up to 12 weeks after treatment against the parasite.

Parasites of elasmobranch olfactory sacs

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Elasmobranchs are commonly infected by metazoan parasites. Their general body surface, eyes, olfactory sacs, mouths, gills, urinary bladders, uterine linings and cloaca all serve as potential habitats for ectoparasites to attach themselves to. Various authors have speculated that the olfactory sacs developed from gill pouches or gill arches, with both gills and olfactory sacs having a supporting structure, i.e. the gill arch or rachis as well as filaments covered with lamellae. Since many copepods are adapted to particular branchial niches it can be expected that some have evolved to occupy the similar niches provided by the olfactory sacs. The parasite infection of the olfactory sacs was investigated. The olfactory sacs of 23 species of elasmobranchs were collected from hosts caught in the nets of the Natal Sharks Board as well as hosts caught by fishermen during fishing competitions at Richards Bay. The tip of the snout containing the nostrils were cut off the host and preserved in 10 % buffered formalin. The olfactory sacs were dissected from the cartilage by which they are encased, preserved in 70 % EtOH and examined for parasites in the laboratory. A total number of 112 hosts were examined. Parasites encountered include monogeneans and copepods (*Kroeyerina scottorum*, *Kroeyerina* sp. 1, *Kroeyerina* sp. 2 and *Nesippus tigris*).

Efficacy of DEET repellents against *Amblyomma hebraeum* (Acari: Ixodidae), the principal vector of *Rickettsia africae* in southern Africa

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African tick bite fever (ATBF) caused by *Rickettsia africae* is an emerging infection in rural sub-Saharan Africa and the French West Indies. Since no vaccination or chemoprophylaxis has proven to be effective, prevention against ATBF relies mainly on personal protective measures against tick bites, including the application of repellents to exposed skin. By using a human bioassay in which repellent-treated fingers were attacked by questing tick nymphs hourly for 4 hours, we tested the repellent efficacy of 4 commercial diethyl-3-methylbenzamide (DEET) lotions against *Amblyomma hebraeum*, the principal vector of *R. africae* in southern Africa. Three commercial lotions with 19.5 %, 31.6 % and 80 % DEET concentrations, respectively, had a repellent efficacy of ≥ 90 % at 1 hour post-application, of < 90 % at 2 hours post-application and of < 70 % during the rest of the experiment. In contrast, a lotion with 2 % DEET plus 1 % citronella oil provided only a 59 % repellency at 1 hour post-application, with a drop to < 22 % during the following hours. Hourly negative control trials repelled < 5 % of tick attacks. The results indicate that commercial repellents containing =19.5 % DEET provide a significant but short-term protection against the tick vectors of ATBF in southern Africa. For maximum protection, the repellents should be applied every 1 to 2 hours.

Autochthonous canine babesiosis in the Netherlands

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Outbreaks of autochthonous babesiosis, caused by *Babesia canis*, occurred in the Netherlands in the spring and autumn of 2004, affecting 23 dogs. Nineteen animals recovered after treatment, whereas 4 dogs died. PCR combined with Reverse Line Blot hybridisation (RLB) revealed that blood samples collected from these dogs were positive for *Babesia canis canis*, whereas no other blood parasites were found. Adult *Dermacentor reticulatus* ticks collected from these dogs indicate that canine babesiosis could become endemic in the Netherlands.

Echinostome parasites in cats in the Fauresmith district, Free State

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Echinostome parasites (Trematoda: Echinostomatidae) are characterised by a collar armed with spines surrounding the oral sucker at the anterior end of the body. One echinostome, *Echinoparyphium recurvatum*, with its 45 spines in the collar, has been described from dogs, cats and humans from Malaysia, Indonesia and Egypt. In South Africa, adult echinostomes have on a few occasions been found in the intestine of fish-eating birds that serve as final hosts. The present study describes an infection of domestic cats with adult echinostome parasites living in the intestine. The cats originated from the Fauresmith district in the Free State Province. They were

sacrificed (Euthapent; Sodium pentobarbitone 200 mg/ml injectable; Kyron Laboratories) to collect gastrointestinal helminthes as part of an endoparasite study. On examination various trematode worms were scraped from the intestines and fixed in 70 % ethanol. In the laboratory at the Medunsa Campus these worms were stained with haematoxylin and studied by light microscopy. Specimens were also prepared for scanning electron microscopy using standard SEM techniques. These intestinal worms measure 3.1–4.5 mm in length and 0.5–0.6 mm in width. They are characterised by a head-collar with 43 spines arranged in a double row, which are likely to cause severe intestinal lesions at the attachment site. This parasite was identified as *Echinoparyphium elegans*. Although the life cycle of this parasite is unknown, *Bulinus tropicus* snails have been found to secrete echinostome cercariae in the Free State. In this specific case, the cercariae most probably infected fish, tadpoles or frogs acting as 2nd intermediate hosts before these free-roaming cats ate them.

A small worm causing great losses in aquaculture

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The family Gyrodactylidae (Monogenea) is probably the most successful group of parasitic flatworms with regard to the number and kinds of animals utilised as hosts. They exhibit a relatively high degree of host, as well as site specificity. Over 400 species of the cosmopolitan genus *Gyrodactylus* have been described and 17 species are currently known from freshwater fish in Africa. Most of these descriptions were from Uganda and Ghana with only 2 species, i.e. *G. transvaalensis* and *G. rysavyi*, described from *Clarias gariepinus*, from South Africa. *Gyrodactylus* causes a condition known as gyrodactylidosis which usually refers to an outbreak of these flukes. Feeding, as well as the haptor with large anchors and marginal hooklets, may cause large wounds, allowing secondary infection. It is their remarkable form of reproduction that contributes to their success. It involves precocious development of intra-uterine embryos, in such a way that worms contain a developing embryo at birth within which a 2nd embryo is already developing and within it a 3rd. This permits fast population growth and greatly reduces generation time. *Clarias gariepinus* were artificially bred and the larvae raised in a 16 m³ recirculating system at 28 °C. The larvae were fed with zooplankton and a balanced dry feed. Three weeks after hatching the larvae congregated at the surface with very little vertical movement. Flow rate of water was increased to compensate for the suspected decreased dissolved oxygen content. Within a day after noticing the decreased movement of larvae, approximately 50 % of the population died. Fish were examined microscopically and gyrodactylids were identified on the skin of all specimens. Within a further 24 h, the rest of the affected larvae died. Owing to the decreased viability of the larvae, it was impractical to execute therapeutic treatment methods. To disinfect the system, 50 ppm of coarse salt was added to water and recirculated through the system for 24 h.

First record of fish blood parasites from the Zambezi River System

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Two fish trypanosome species have been described from various localities in southern Africa. No literature exists on the presence of these blood parasites in fishes from the Zambezi River, Caprivi. During 2004, 2 species of catfish, *Parauchenoglanis ngamensis* and *Synodontis nigromaculatus*, were caught with rod and reel and transported back to the University of Johannesburg's aquarium. In the aquarium, blood was drawn from the fishes' caudal veins, smeared on clean microscope slides, fixed for 5 minutes in methanol and stained for 25 minutes with Giemsa's stain. Trypanosomes found in the blood smears were photographed and measured with the aid of a Zeiss AxioPlan compound microscope and Axio Vision 4.2 software. It was found that both specimens of *P. ngamensis*

were infected with trypanosomes, with 1 having a very high infestation (more than 100 trypanosomes per blood smear). Different life stages of trypanosomes were observed in this highly infected fish. Seven of the 13 *S. nigromaculatus* studied were also found to harbour trypanosome infections. None of these fish had such a high infection as was found in the *P. ngamensis*. When compared to existing literature, it was clear that the size range of the trypanosomes found in these catfishes correlated with that of *Trypanosoma mukasai*, which had been described from the same hosts from the Okavango Delta, Botswana. This is the 1st record of fish trypanosomes from the Zambezi River System.

Detection of multiple tick-borne pathogens in cattle at Ficksburg and Reitz, eastern Free State, South Africa using reverse line blot hybridisation

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Ticks and tick-borne pathogens are responsible for mortality and morbidity in livestock, wildlife, pets and humans. In cattle, tick-borne pathogens such as anaplasmosis and babesiosis cause diseases of worldwide economic significance. However, detection of these pathogens is often difficult with multiple infection cases and low infection rates in carrier animals. Reverse line blot hybridisation was used to survey the prevalence of tick-borne pathogens in cattle on commercial and small-scale resource-poor farmers in the eastern Free State. Blood was collected from 74 cattle blood samples not showing clinical symptoms on 5 farms at Ficksburg and Reitz. DNA was extracted and subjected to 2 different PCRs: an 18S rRNA-based PCR for amplification of *Theileria* and *Babesia* species and a 16S rRNA-based PCR for amplification of *Ehrlichia* and *Anaplasma* species. Then a reverse line blot hybridisation was performed with family and species-specific DNA probes. Forty-nine per cent of the samples were infected with *Anaplasma marginale*, 35 % with *Theileria taurotragi*, 18.9 % with *Ehrlichia* sp. Omatjenne, 2.7 % with *Babesia bigemina*, as well as 1.4 % with *Anaplasma bovis* and *Babesia bovis*. These results indicate a situation approaching endemic stability with moderate risk of clinical anaplasmosis on the sampled farms. It is concluded that on these farms there is a fairly high tick challenge or a combination of tick and biting flies challenge which means that there are low levels of tick control and possibly little use of acaricides.

Piroplasmiasis as a possible cause of mortality in giraffes

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Two cases involving adult giraffes, from different geographic areas, showed sudden onset of disease. The course of the disease in each case was peracute and appeared to affect many organ systems. Some of the clinical signs noted were: severe depression, recumbency and severe weakness, hypothermia, dyspnoea, rumen stasis and oedema of the sclera and conjunctiva. Some of the *post mortem* findings included frothy nasal discharge, haemoglobinuria and haemopericardium. Microscopic examination of thin blood smears revealed the presence of small piroplasmids. In an attempt to confirm piroplasmiasis as the cause of disease, DNA was extracted from blood and spleen samples. The V4 variable region of the 18S rRNA gene was amplified and analysed using the Reverse Line Blot (RLB) assay. RLB results indicated that the PCR products did not hybridise with any of the *Babesia* or *Theileria* species-specific probes present on the blot, but only hybridised with the *Babesia/Theileria* genus-specific probe. The full-length 18S rRNA genes were subsequently amplified and cloned using the Promega pGEM T Easy Vector System. Recom-

binants were further analysed by sequencing analysis. Sequencing data were analysed and edited with the Staden package and aligned with published sequences of related genera using the ClustalX software programme. Phylogenetic trees were constructed using the neighbor-joining method in combination with the bootstrap method. The sequence similarity analysis indicated that a *Babesia* species infection was present in the 1 giraffe and a *Theileria* species infection in the other.

Bartonellae: arthropod-borne parasites in small terrestrial vertebrates from South Africa

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Bartonella species are able to invade and persist in erythrocytes and thus chronically infect the blood of their hosts. These bacteria emerged as pathogens of humans and domesticated animals. Fewer than 30 mammal species have been surveyed to date, and thus it is likely that the true extent of variety within the genus is far greater than is currently recognised. In this study we have attempted to extend this exploration by studying bartonellae associated with small mammals endemic to South Africa. Small terrestrial mammals were trapped, identified, euthanased and blood was collected aseptically by cardiac puncture. Attempts were made to isolate *Bartonella* species, then PCR and sequence analysis were performed. Electron microscopy was also performed on isolates. A total of 10 different rodent species were trapped. Blood samples ($n = 86$) yielded 38 cultures of putative bartonellae within 2 weeks of incubation. The overall prevalence of infection was 44%. Partial *gltA* fragments of 689 bp were obtained for 32 isolates. Phylogenetic analysis revealed that the isolates yielded 24 different sequences within 2 clusters, 1 unique to South Africa. This was the 1st study to confirm the presence of bartonellae in rodents endemic to southern Africa and the 1st to investigate the diversity of organisms associated with these infections across the entire African continent.

***Rickettsia aeschlimannii* and *R. mongolotimonae*: arthropod-borne pathogens of medical importance in South Africa**

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Until very recently, Mediterranean spotted fever caused by *Rickettsia conorii* was the only spotted fever group (SFG) rickettsiosis recognised in southern Africa. African tick bite fever (ATBF), caused by *R. africae*, is currently the most important of these new rickettsioses, affecting large numbers of international travellers each year, but infections due to *R. aeschlimannii* and *R. mongolotimonae* have also been recently encountered. Two case reports are described, together with the current status of the epidemiology, clinical presentation, diagnosis and treatment of SFG rickettsiosis in southern Africa. Samples of patients were tested using serology, PCR and sequence analysis. Two new pathogens, *R. aeschlimannii* and *R. mongolotimonae*, belonging to the SFG rickettsiae, were identified in South Africa. At least 4 SFG rickettsioses are endemic to southern Africa. While ATBF seems to be the most widespread and most commonly occurring, notably among visiting international travellers, severe and life-threatening cases are virtually only seen in MSE. Clinicians should know that specific and sensitive diagnostic techniques are available to differentiate between the infections. Further studies are needed to define more clearly the relationship among *R. conorii*, *R. africae*, *R. aeschlimannii* and *R. mongolotimonae*, and in particular their geographical distribution and clinical presentation of infection.

Impact of water quality on abundance of parasites from *Oreochromis mossambicus* at the Phalaborwa Industrial Complex (PIC) and Barrage: preliminary results

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Fish parasites are believed to cause little or no harm to their hosts under natural environmental conditions. Chronic exposure to pollutants may cause biochemical, physiological and behavioral host changes that ultimately can influence the intensity and prevalence of parasites. Contaminants in water may increase parasitism if the host defense mechanisms are negatively affected, thereby increasing host susceptibility. However, pollution can also decrease parasitism if the parasites are more susceptible to particular pollutants than the host, or pollution levels eliminate the suitable intermediate host. This project involves seasonal sampling of 3 sites at the PIC and 1 site at the Phalaborwa Barrage (Olifants River). The metazoan parasites of *Oreochromis mossambicus* were recorded for the winter season thus far. Water qualities were determined at all the sites. Parasites were fixed and preserved using standard methods. A parasite index (PI), abundance, prevalence and mean intensity of parasites were calculated. The results indicate that the water quality is poor at 2 sampling sites of the 2 mines caused by non-toxic constituents (chlorides, magnesium and sulphates) while the pH and TDS of the Barrage and fertiliser plant differed significantly from the other sites. The metal concentrations were acceptable at all sites, except for zinc and potentially lead. The following ectoparasites were recorded: *Cichlidogyrus* spp. from the gills and *Lernaea cyprinacea* from the skin. Endoparasites included digenean larvae ('black spot') from the skin, *Clinostomum* larvae in the cranial cavity, dilepidid cestode larvae from the liver and intestine, *Contracaecum* larvae from the body cavity and digenean and pentastomid larvae from the swim bladder. The lowest numbers of ectoparasites were encountered at the Barrage and fertiliser plant in correspondence with our hypothesis and the lower non-toxic constituents.

Myxosporean parasites in freshwater fish of the Okavango Delta, Botswana

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Fish that occur in warm water provide a niche for a diversity of parasitofauna. One of these groups of parasites belongs to the class Myxosporidia. Myxosporidia are minute endoparasites of which their most obvious characteristic is the production of many tiny spores at certain stages of their life cycle. Myxosporidia are capable of infecting any organ and may be divided into 2 groups based on the site preference within the host. Coelozoic species live in body cavities such as gall- or urinary bladders and histozoic species are found within various tissues. The spores are characterised by the presence of 1 to 4 polar capsules and polar filaments (attachment device) within the capsules. The project's objectives comprise investigating myxosporidian species invading the internal tissue and organs of fish and assessing their taxonomic status. Fieldwork involved the collection of fish from the river systems using handsets, gill nets, rod and line. Internal organs of anaesthetised fish were collected and compressed between 2 glass slides. The liquid media were examined for spores using light microscopy. Material was also fixed in Glutaraldehyde for Scanning Electron Microscopy. Large myxosporidian cysts of the genus *Myxobolus* were found in the buccal cavity of *Pseudocrenilabrus philander* and ovaries of *Clarias gariepinus*. Histozoic myxosporidia also of the genus *Myxobolus* were found in the liver of *Hydrocynus vittatus*, in the spleen, kidneys, and ovaries of *Synodontis nigromaculatus* and ovaries of *C. gariepinus*. Myxosporidia of the genus *Henneguya* were found in the spleen of *Scilbe intermedium* and gill lamellae of *C. gariepinus*. These findings indicate that many more fish species are probably infected with histozoic myxosporidia and through continuation of this project, these unknown species will be described morphologically and taxonomically.