

Parasitological Society of Southern Africa

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Keynote Addresses

Taking a 'tryp' across the human blood–brain barrier: a new adventure

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African trypanosomes are major pathogens of humans and animals. *Trypanosoma congolense* and *Trypanosoma vivax* are pathogens of cattle but do not infect humans. Two subspecies of *Trypanosoma brucei*, *T. b. rhodesiense* and *T. b. gambiense*, are human pathogens while the closely related subspecies *T. b. brucei* is non-infective to humans. The latter is frequently used as a model for trypanosomiasis in laboratory animals. Each of the above parasites plays an important role, sometimes devastating in the health and welfare of people and cattle throughout large areas of sub-Saharan Africa. Although there were indications that human trypanosomiasis (commonly called African sleeping sickness) was under control during the middle of the 20th century, the World Health Organization reports that several countries are seeing a resurgence of trypanosomiasis of epidemic proportions. Because death is inevitable if a patient is untreated, human trypanosomiasis has been claimed to be more deadly than other vector-borne diseases, such as malaria.

In its terminal stage, human sleeping sickness is characterised by muscle wasting and a general increase in nighttime insomnia and daytime drowsiness. Because the neurological symptoms of human sleeping sickness frequently occur late in the course of the disease, *i.e.* just prior to death, it is often assumed that the invasion of the CNS occurs late in the course of the infection. However, *T. b. rhodesiense* can be found in the cerebrospinal fluid (CSF) of patients as early as 7–10 days after commencement of illness. Early infections of the CNS have also been seen in laboratory animals. Interestingly, parasites that are injected directly into the brain do not survive and the CSF alone is insufficient to maintain the parasites *in vitro*. Yet, the parasites that cross the BBB *in vivo* remain viable. Once inside the brain the parasites are safe from many of the most effective trypanocidal drugs. Indeed, the brain is probably the source for many relapse infections. Consequently, if the parasites do cause any damage to the barrier, such damage must be either minimal or transient.

Despite its importance, the mechanism by which the trypanosomes enter the CNS remains an unresolved issue. *In vitro* models of the BBB have clearly become important tools for identifying the cellular and molecular elements that may be possible targets for interventions for the transmigration of many pathogens into the CNS. Because it is not possible to undertake *in vivo* experiments, an *in vitro* model of the BBB is essential if we are to understand how trypanosomes cross the human BMEC that comprise the functional unit of the human BBB. Using an extensively tested *in vitro* model for the human BBB developed by this laboratory, we examined how African trypanosomes transverse the human BBB.

We tested the ability of *T. b. brucei* TREU 927 (*Tbb* 927) [the *T. brucei* genome reference strain], *T. b. brucei* 427 (a rodent adapted laboratory strain), or human infective *T. b. gambiense* IL 1852 (*Tbg* 1852) to cross the BBB. When *Tbb* 927 and human infective *Tbg* 1852 were incubated overnight with human BMEC grown on Transwell™ inserts, the data from several experiments revealed that approximately 20–30 % of the total *Tbg* 1852 bloodstream form (BSF) parasites were able to cross the human BMEC from the upper to lower compartments compared to <5 % for BSF *Tbb* 927. Insect infective procyclics do not bind or cross human BMEC, even when co-incubated with BSF *Tbg* 1852. Although there was little or no change in TEER when human BMEC were incubated with *Tbb* 927, human BMEC traversal by *Tbg* 1852 is accompanied by a statistically significant 15–18 % drop in TEER relative to the BMEC controls without

parasite. In a kinetic study, using BSF parasites that were fluorescently tagged with PKH67 (an FITC-like cell tracker dye), we were unable to detect *Tbb* 927 BSFs after 6 hours. In addition, more *Tbg* 1852 crossed the model barrier in 3 hours than did the PKH67-labelled *Tbb* 927 parasites in 16 hours: 7 % versus 2 %, respectively. Data for *Tbb* 427 were essentially the same as for *Tbb* 927. When BMEC was omitted from the Transwell filter, more than 80 % of the parasites migrated into the lower Transwell chamber, indicating the trypanosomes are able to freely cross the Transwell filter and that the human BMEC indeed formed a true barrier.

We also determined whether GPCR-mediated PLC calcium responses in human BMEC induced by African trypanosomes is linked to the parasite's transmigration across the BMEC barrier. It is well known that activated PLC splits PIP₂ to yield IP₃ and diacylglycerol (DAG). IP₃ causes release of Ca²⁺ from intracellular stores (*i.e.* ER), which in turn activates protein kinase C (PKC), an event that also requires DAG binding. The transmigration of *Tbg* 1852 across human BMEC grown on Transwell™ inserts was inhibited when the BMEC were pretreated for with U73122, a PLC inhibitor, prior to washing and addition of the parasites. While trypanosome crossing of human BMEC was observed, no parasites were detected in the U73122-pretreated human BMEC. We also monitored Transwell™ TEER using the EndOhm chamber over the course of the experiment. Pretreatment of BMEC with U73122 tightened the barrier to the trypanosomes. In line with the known function of PLC, the inhibition of trypanosome migration across human BMEC pretreated with a [Ca²⁺]_i chelators or PKC inhibitors, lends further support for PLC's role in BBB transmigration by African trypanosomes.

Comparative micromorphological specializations of the Phthiraptera (lice) as revealed by scanning electron microscopy

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Scanning electron microscopy (SEM) has become a major tool used in the description of new species of arthropod ectoparasites during the past 3 decades, due to the greatly increased depth of field and magnification of SEM compared to light microscopy. In this paper a number of morphological characteristics that have traditionally received little attention will be presented, as well as a number of new micromorphological characteristics only visible by SEM but which may have taxonomic importance in future studies of lice.

The lice were collected live and fixed in 70 % ethanol. The specimens were cleaned by ultrasonication, routinely processed; sputter-coated and viewed in a Leica Stereoscan 420 SEM at 5 to 10 kV.

The complex specializations of the heads of the Mallophaga with their biting, chewing or rasping mouthparts were compared with those of Anoplura having simple sucking mouthparts. The structure of the antennae was often sexually dimorphic with the male antennae having specializations for grasping the females during copulation. The structure and position of the main types of antennal sensoria, including the pore organs, tuft organs, plate organs and peg organs, were identified and compared by means of SEM. Differences between these sensoria were often shown to be species-specific even in morphologically closely related species.

As obligatory ectoparasites, it is essential for the lice to remain attached to their hosts. The legs and mandibles of the bird lice showed interesting specializations for holding on to different feather types while the legs of the Anoplura were specialised for attaching to the different hair types of their mammal hosts. Setae on the body were similarly adapted for hooking into the fur to prevent dislodging during grooming.

Micromorphological differences between lice of various families were also illustrated by the different integumental surface struc-

tures ranging from membranous to scales and protective plates. The distinctive internal surface structure of the spiracles of each of the families reflected different structures which effect the common function of filtering the air entering the tracheae of different groups of lice.

The SEM studies on the structure of the female genitalia usually confirmed the accepted light microscopic descriptions but with much greater clarity. However, when the everted male genitalia were present, the SEM shed new light on the uniqueness of the micromorphology of these species-specific organs, as well as the process of how the parameres and pseudopenis unfold outside the genital opening.

This paper illustrates how valuable the SEM is in the examination of micromorphological specializations and the interpretation of the functional morphology of lice as well as in the ongoing search for new taxonomic associations.

Oral Presentations

Species specificity in Diplozoidae in freshwater fishes in Vaal Dam

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The majority of Monogenea are known to be extremely host specific, to such an extent that their presence is used to identify hosts or to track translocations and introductions. Diplozoidae were collected from gills of fishes in the Vaal Dam. The fishes were collected with gill nets and identified using gross morphological characteristics. They were killed prior to removal of gills. The gills were scrutinised for the presence of parasites. Parasites were fixed and stained with a variety of stain solutions. Parasites were present on the yellow fishes *Labeobarbus aeneus* and *Labeobarbus kimberleyensis* as well as on *Labeo umbratus*. Previously, other researchers showed that the 2 yellow fish species may be inbreeding. To identify the parasites the structure of the opisthaptor, the shape and structure of the attachment clamps, the position of the reproductive organs, annulations on the posterior part of the body and the shape of the eggs were studied. Two morphological types were distinguished. It was shown that the Diplozoidae from the 2 yellow fish species were morphologically similar although the habits and habitats of the hosts were supposedly different. The 2nd parasite type was present only on *Labeo umbratus* although *Labeo capensis* was also present in this impoundment. This case may therefore indicate that the yellow fishes are closely related or have been breeding in. Another explanation in this case may be that the parasite was able to survive on more than 1 species or even that the characteristics employed for taxonomy in this group was inadequate.

Protozoan fish parasites and their implication in aquaculture in the Okavango Delta, Botswana

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The Okavango River and Delta, located in the northwestern part of Botswana, harbour some 70 species of fish. During parasitological surveys carried out during the last 6 years, 67 species of wild fish were investigated for the presence of ectoparasites. Several protozoans were encountered on the skin, fins and gills, the majority of which belonged to the phylum Ciliophora. These included many trichodinid species, sessiline species, as well as *Chilodonella* Strand, 1926, species, *Ichthyophthirius multifiliis* (Fouquet, 1876) and a few suctorian species. A single flagellate (phylum Mastigophora) was encountered as well, i.e. *Ichthyobodo necator* (Henneguy, 1883). The Okavango River does not contain any introduced or translocated fish in its system, although many of the ectoparasites encountered are known worldwide as potentially dangerous under culture conditions. Aquaculture is still in its infancy in Botswana, but the results obtained to date indicate that there are several parasites that are pathogenic under culture conditions. In 1 venture, we have already encountered several protozoans where these were responsible for serious mortalities of cichlids.

Aspects of the ecology of the Asian tapeworm, *Bothriocephalus acheilognathi* Yamaguti, 1934, in yellowfish in the Vaal Dam, South Africa

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Seasonal surveys were conducted at the Vaal Dam between April 2000 and January 2001. During each survey twenty smallmouth yellowfish (*Labeobarbus aeneus*) and twenty largemouth yellowfish (*Labeobarbus kimberleyensis*) were collected with the aid of gill nets. Surface water quality variables were included. Cestodes were identified as either *Bothriocephalus acheilognathi* Yamaguti, 1934, or 'other cestode spp.'. The majority (99.8%) of cestodes found in both yellowfish species were identified as *B. acheilognathi*. The prevalence, mean intensity and abundance of *B. acheilognathi* (Asian tapeworm) in both yellowfish species were calculated. Ecological parameters including species specificity, seasonality, gender specificity and relationships between fish size and Asian tapeworm prevalence were also included. In this study *B. acheilognathi* preferred *Lb. kimberleyensis* over *Lb. aeneus* although a low intensity was observed in smallmouth yellowfish. Furthermore the infection (in terms of prevalence, abundance and mean intensity) in largemouth yellowfish was markedly higher. Seasonal patterns observed in the Asian tapeworm's infection of smallmouth yellowfish were attributed to breeding and subsequent feeding patterns of this fish species with relatively high infections recorded in winter and spring. No explanation could be given in the case of *Lb. kimberleyensis* regarding the seasonal patterns observed for the mean intensity and abundance of *B. acheilognathi*. The maximum and minimum mean intensity and abundance values in largemouth yellowfish were recorded in autumn and spring, respectively. In addition the prevalence of *B. acheilognathi* was consistently high in all 4 seasons. It is therefore deduced that either a 2nd intermediate host is involved or that the intermediate host can vary considerably.

New *Babesia* parasites in cheetahs and other wild felids

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Babesia, an intracellular erythrocytic haemoprotezoan parasite of mammals, has also been reported in reptiles and birds. A diagnostic assay, the Reverse Line Blot (RLB) hybridization technique is currently used to detect *Babesia* and other blood parasites in a variety of animal species. Results obtained from this test showed that there might be undescribed *Babesia* parasites other than *B. felis* and *B. leo* present in wild felids.

Specimens from the field are submitted directly to our laboratory by various collaborators. In addition, specimens submitted to the Clinical Pathology Laboratory that are found to harbour piroplasms are forwarded to our laboratory for further processing.

DNA was extracted from domestic cats (*Felis domesticus*); lions (*Panthera leo*); cheetahs (*Acinonyx jubatus*); black-footed cats (*Felis nigripes*) and servals (*Felis serval*) using the QIAamp DNA extraction kit. Polymerase chain reaction (PCR) was performed using primers that amplified a variable region of the 18S ribosomal rRNA gene. The PCR products were analysed using the RLB technique. The parasites that tested positive with only the genus-specific *Babesia/Theileria* probe were cycle sequenced, using an ABI PRISM BigDye™ Terminator v3.0 Ready Reaction Cycle Sequencing Kit. Electrophoresis of the sequencing reactions was done on the ABI 310 automated sequencer, according to instructions of the manufacturer (Applied Biosystems). The sequencing data were analysed using the Staden package.

Results showed that *B. felis* occurred in both lions and domestic cats and can occur concurrently with other haemoparasites. *Babesia* was also detected in cheetahs, a black-footed cat and in a serval, but the species could not be determined. Sequencing data from the parasite in cheetahs differed from the known *B. felis* and *B. leo* sequences (Genbank), but closely resembled that of the *B. gibsoni* group of organisms.

These results indicated the presence of a possible new *Babesia* parasite in cheetahs, but these results must be still confirmed by phylogenetic analysis and serology.

Screening of 4 anthelmintics for their efficacy against the gill-parasitising monogeneans *Calceostoma* sp. and *Diplectanum* sp. from the silver kob, *Argyrosomus inodorus*

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Finfish mariculture in South Africa currently revolves around 2 species namely *Argyrosomus inodorus* and *A. japonicus* (the silver kob and dusky kob, respectively). Various parasitic helminth taxa are continually restricting efforts to domesticate and acclimate both wild-caught dusky and silver kob as brood stock in pump ashore systems. Of particular importance are representatives of the Monogenea, which have a direct life cycle and as such require no intermediate host and can repopulate or re-establish an infestation in remarkably short periods of time in captivity. Representatives of 2 monogenean genera, namely *Calceostoma* sp. and *Diplectanum* sp., are of particular importance and have been responsible for mortalities among both *A. inodorus* and *A. japonicus* in captivity at various localities along the South African southern coastline. Four chemicals, *i.e.* formalin, hydrogen peroxide, Praziquantel and Mebendazole, were screened for their efficacy against both *Diplectanum* sp. and *Calceostoma* sp. on *A. inodorus*, respectively. These chemotherapeutants are routinely used for the treatment of monogenean infestations on captive fish globally. Only formalin showed a significant reduction in parasite numbers of both *Diplectanum* sp. and *Calceostoma* sp. but did not eradicate either population from the gills of the fish. Hydrogen peroxide was effective in totally removing *Calceostoma* sp. from the gills of the host but did not show any significant reduction in the number of *Diplectanum* specimens from the hosts. Both commercial anthelmintics screened showed no significant effect on either monogenean population.

Attachment of *Nesippus orientalis* (Siphonostomatoida: Pandaridae) specimens to their hosts

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Many species of adult copepod parasites infecting elasmobranchs are found attached at particular sites on their hosts. In fact, this site specificity goes beyond the general habitat descriptions of gills, mouth, body surface, etc. Considering species found on the gills, as an example, there are those found exclusively on the arches (*Pandarus* spp.), those attached to the interbranchial septa of the gills (*Phyllothyreus cornutus*, *Paeon* spp.), those that attach to the tissues surrounding the efferent arterioles of the gill filaments (*Nemesis* spp.), those that live in the excurrent water channels between gill filaments (*Kroyeria* spp.) and those that attach to the secondary gill filaments (*Eudactylinodes* spp.). In order to utilise different sites effectively, species adapt variously to attach and feed at the specific sites.

Specimens of *Nesippus orientalis* were collected from the gill arches and mouths of a variety of shark hosts at the facilities of the Natal Sharks Board, KwaZulu-Natal. They were preserved in 70 % EtOH and selected specimens were examined using stereo- and scanning electron microscopy. Individuals of *N. orientalis* often align themselves on the gill arches so that an orderly arrangement of clustered individuals in staggered rows is achieved. In such a configuration the genital segment of 1 individual overlaps the cephalothorax of the next. Scanning electron micrographs of a specimen detached from the gill arch of the host while still grasping a placoid scale reveals how the maxillipeds of this species are used to attach to the host.

Some responses of salivary glands in female *Hyalomma marginatum rufipes* to water and garlic

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During the initial part of this investigation it was evident that garlic elicits a response by the salivary gland acini of feeding adult females of *H. m. rufipes*. However, some uncertainty remained as to the effects of crude garlic extracts on the acini of tube-fed females of this species. The low activity of diaphorases in experiments targeted at determining co-enzyme support for enzyme systems also needed further investigation. It was the aim of this study to continue the investigation, 1st to revisit the effect of crude garlic on the salivary gland acini of tube-fed females of this species during the 1st 3 days of feeding, and 2nd to duplicate the diaphorases experiments in order to determine the validity of the initial experimental results. To date, material has been collected from 76 flat adult females of *H. m. rufipes* (64 in the 1st trial + 12 during the follow-up trial). During host feeding, half the hosts received a crude garlic extract as water supply and the others water without the garlic addition. On the other hand, in the tube-feeding programme, half of the ticks were fed with the garlic extract while the rest were fed with pure water. The salivary glands that were removed from each group were pooled in neutral phosphate buffer. Samples were taken from each pool for histochemistry, routine histology and transmission electron microscopy. Duplication of the tube-feeding trial as well as the diaphorases experiments confirmed the findings of the initial investigation: no distinct difference was observed between acini of the 2 groups. Ticks did feed on both solutions (as indicated by an increase in mass) and the average diameter of the salivary gland acini, which increased during the early part of feeding in both groups, was not convincingly different (on average the recorded acini diameters of garlic-fed ticks were similar, only slightly larger in garlic-fed than in water-fed ticks). A comparison of TEM information, on both the mitochondrial distribution and the nature of the ER, to the diffuse reaction product recorded in the NADH-diaphorases experiments, suggest activity of this co-enzyme outside the mitochondria. Mitochondrial distribution as well as the involvement of the rough ER in diaphorases activity needs closer scrutiny.

Functional micromorphological specializations of the human body louse

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Human body lice are ectoparasites infesting human clothing in which they lay eggs but return to the body of their host for their blood meals. Besides the irritation caused by their bites, these lice are vectors of potentially lethal diseases such as epidemic typhus and relapsing fever in man. This study was aimed at investigating the micromorphology of these lice by scanning electron microscopy (SEM) in order to obtain some understanding of how these lice attach to, feed and orientate between their hosts and their clothing. Body lice collected in Lethbridge, Alberta Canada, were fixed in 70 % ethanol. After ultrasonic cleaning, the lice were routinely prepared and viewed in a Leica Stereoscan 420 SEM at 5 to 10 kV.

The head included a pair of lateral eyes, each of which was covered by a simple lens, a pair of 5 segmented antennae and a single tubular haustellum. Short tooth-like setae were observed on the flaps forming the suckerlike haustellum which housed the hooked stylets used for penetrating the skin to reach the underlying blood vessels. The distal antennal segment 5 bore most of the specialised sensoria including a tuft organ protruding from a pore organ, a double plate organ and the terminal peg organ consisting of 10 sensillia. Segment 4 only had a pore and tuft organ. These sensoria are required to orientate between the body and the clothing, to locate prospective mates and retreat from noxious substances. The thorax was fused for strength, with a pair of legs attached to the pro-, meso- and metathorax segments. Each joint of the leg had a scaled membranous area with a number of gland-like openings. Each leg terminated in a tarsal claw which closed against the distotibial process

with its spinous setae. The tarsal apophysis bore a long, curved spine which further improves attachment to both body hair and clothing fibres. A large round spiracular opening was seen on the mesothorax which was lined internally by honeycomb-shaped lamellae which may act to filter the air entering the trachea. The 6 pairs of abdominal spiracles were embedded in the lateral protective paratergites. These protrusible spiracles may enable the louse to regulate the entrance of toxic substances such as insecticidal shampoos. The abdominal integument was flexible allowing for extension during feeding. Gonopod VIII of the female is designed to clasp the clothing fibres while the long tactile setae of gonopod IX holds the egg in position while it is cemented to these fibres. These specializations have contributed to these lice being successful human ectoparasites for millennia.

Micromorphology of sucking lice from Thompson's gazelle

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The only description of sucking lice collected from a Thompson's gazelle was made by Bedford in 1934 from 3 female lice which he named *Linognathus lewisi*. The present study sought to investigate the micromorphology of these lice by scanning electron microscopy (SEM) in order to understand how they attach to, feed and orientate on their host, as well as describing the males for the 1st time. The lice were collected from Thompson's gazelles by a veterinarian in Dubai, and fixed in 70 % ethanol. After ultrasonic cleaning, the lice were routinely prepared for SEM and viewed in a Leica Stereoscan 420 SEM at 5 to 10 kV.

The elongated heads were eyeless, but had prominent ocular points, which are also characteristic of *L. africanus*. Long tactile setae (8 + 4) surrounded the sucker-like haustellum. The distal antennal segments bore most of the specialised sensoria including a large pore organ, 2 small plate organs and the terminal peg organ consisting of 10 sensillae while segment 4 only bore a large pore organ. These sensoria are required for orientation on the host. The legs were specialised attachment organs with the legs II and III bearing robust tarsal claws. These claws closed against double distotibial pads covered with scales to increase the grip on the hairs. There were no distotibial spinous setae, but each claw fitted into the groove between these distotibial pads. These claws clasped snugly around the flattened hairs of their host. The 1st pair of legs only had slender claws and the pads were also reduced in size.

The abdominal integument was membranous but covered with overlapping scales and lanceolate setae. The spiracles included a pair of large thoracic spiracle and 6 pairs of abdominal spiracles, each enclosed by a spiracular plate and lined internally by a number of subsurface rings. These rings may act to filter the air entering the tracheae. The abdomens of the males were shorter and broader than those of females, while the posterior 3 segments were deeply infolded. The terminal gonopods of the males were cone-shaped and directed dorsally. In the female lice the shape of the subgenital plate and gonopod VIII including their arrangement of setae observed in this study, differed from the original description by Bedford. The differences may result from observations made on cleared specimens on a slide compared to more natural specimens observed in the SEM, or alternatively the specimens included more than one *Linognathus* species.

Although many of the morphological characteristics of the females corresponded with those described by Bedford for *L. lewisi*, some of the lice were positively identified as the African blue lice *L. africanus* that commonly occur on African goats. This study confirmed Thompson's gazelle as a new host for *L. africanus*.

Dispersal of ticks and tick-borne diseases by birds

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The population dynamics of ticks (Acari) is dependent on climatic

factors, vegetation, abundance of suitable hosts for the different instars, predation, pathogens, human use of the landscape and dispersal. Ticks have a very limited own dispersal ability, but may be transported over long distances by the vertebrates they parasitise, even across geographical barriers like deserts or seas. Our purpose is to quantify birds' role in spreading ticks.

Climate data seems to be the best predictor of tick species distribution. In Norway we have had 7 cases of TBE during the last 5 years, a previously unobserved disease. A climate model predicts occurrence of TBE in Norway, and an increasing distribution of the disease in Scandinavia.

Birds were trapped with mist nets during spring migration 2003 and 2004 at 4 bird ringing stations along the south coast of Norway. They were examined for tick infestation, and the ticks were preserved in 70 % ethanol. The birds were classified as 'newly arrived' if large numbers of 1 species suddenly occurred in the nets. Ticks from newly arrived birds, which contained blood, were selected for DNA examination. DNA was extracted by DNeasy spin columns where after detection of pathogens was performed by PCR.

In 2003 we found ticks on 8.9 % of the all the examined birds, and on 5.8 % of the presumed newly arrived ones. In the ticks examined for pathogens we found *Anaplasma* sp. in 4, *Babesia divergens* in 3 and *B. garinii* in 1 of the 89 examined ticks.

Identification of protective antigens from the midgut of *Amblyomma hebraeum* (Acari: Ixodidae)

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This study demonstrates the involvement of midgut proteins in the acquisition of resistance to *Amblyomma hebraeum*. Here rabbits were immunised with midgut antigens, and then later challenged with *A. hebraeum* adult ticks. Three groups of rabbits were used for this purpose; 2 were immunised with midgut extract, 1 with adjuvant and 1 was never immunised. Humoral immune responses of rabbits to tick antigens were quantified by the dot blot procedure before and after tick challenge. The results showed that rabbits immunised with midgut extracts had antibodies against *A. hebraeum* midguts which increased after tick challenge. Rabbits immunised with adjuvant responded only after tick challenge, whereas those which were never immunised did not respond at all. In the follow-up experiment, ticks collected from 3 groups of rabbits mentioned above were observed for feeding and reproductive performances. After feeding, the number of ticks which died and those that engorged were recorded. Engorged ticks were weighed, cleaned and placed in glass vials for oviposition. After oviposition, egg mass and percentage hatchability were determined. The findings revealed significant impaired feeding in terms of reduced tick yield and engorgement weights of *A. hebraeum* female ticks. Engorgement weights of ticks which fed on immunised rabbits were reduced by 40–54 %, whereas only 80 % feeding success and 74 % egg hatchability were recorded. Twenty per cent of adult ticks which did not feed successfully on immunised rabbits and later died *in situ* were recorded. Mortality of ticks which engorged on immunised rabbits amounted to 10 %. Western blot analysis of midgut proteins was done after midgut extracts were run on an SDS-PAGE and transferred to a PVDF membrane for hybridization. The results showed that 2 proteins might be responsible for conferring immunity in rabbits immunised by *A. hebraeum* extracts. The 2 proteins which were revealed are of molecular weight 85 and 145 kDa. Future studies will involve characterization of the *A. hebraeum* 85 and 145 kDa proteins and subsequent cloning, sequencing and expression of these proteins.

Missed diagnoses of schistosomosis (bilharzia)

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Though bilharzia is prominent in Africa, it often remains undiagnosed. This parasitic disease is caused by a trematode of the genus

Schistosoma. Bilharzia infects about 200 million people worldwide, killing 250 thousand annually. Amongst my CFS patients, presenting with rickettsial, chlamydial and/or mycoplasma infection, I routinely perform a serological investigation for bilharzia.

Bilharzia is carried by snails. Adult worms swim in the blood vessels. The anatomical location of the parasites will dictate different symptoms. If located in the central nervous system, schistosomiasis causes grand mal epilepsy. When *Schistosoma* migrates to the small intestine or the colon, chronic diarrhoea and polyps can occur. Heart failure begins after penetrating the lungs. The skin is another target. In addition to this, *Schistosoma* invades muscles. After a while, hepatomegaly and splenomegaly are commonly seen.

All of the above diverges from the standard portrayal of bilharzia: Charting the above itinerary of bilharzia is enough to confuse the best physician.

It is clear that 1 microorganism can be the start of different diseases depending in which organ it is located and conversely the same disease may also be produced by different microorganisms. The difficulty lies in the diagnosis and the prognosis depends on early diagnosis.

In Africa, I cannot emphasise the use of Biltricide (praziquantel) strongly enough every time the disease is suspected (even though the diagnosis may be clouded by an absence of biological evidence).

Is parasite diversity an indication of host niche adaptation?

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The major factor determining the distribution of fish within the Okavango Delta appears to be habitat preference with physical barriers playing a minor role. During surveys carried out in the Okavango River and Delta over a period of 5 years, 67 of the 68 fish species known were collected and examined. These specimens included 6 species from the genus *Synodontis*. Parasites from these fish included monogeneans, crustaceans and a trichodinid from the gills and skin, respectively, as well as digeneans, ciliophorans and nematodes from the alimentary canal. The parasites were fixed according to standard methods for each group of parasite for further microscopical studies. The *Synodontis* species collected were found in different habitats within the river and have different feeding strategies. The spotted squeaker, *Synodontis nigromaculatus*, prefers rocks or marginal vegetation and feeds on detritus, algae, plant material, snails and small fish, while the finetooth squeaker, *Synodontis vanderwaali* does not feed on plant materials and algae at all. These fish could therefore be used as a classical example of adaptive radiation. Each *Synodontis* species has its own set or group of parasites, some of these parasites being common to all the species, while others are unique to a specific fish host (especially the fauna of the alimentary canal). It is therefore possible that this niche adaptation of the host might give an indication of parasite diversity.

Patients with inexplicable complaints of parasite infestation of the skin: differential diagnosis

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Investigation of inexplicable complaints of parasite infestation of the skin should include a thorough physical examination of the patient to ensure that no underlying medical problems exist and that, in fact, no parasites are present. In addition to lice and *Sarcoptes* spp., there are a number of mites and insects that are known to bite humans and cause irritation and itchy patches on the skin. These include species of the genera *Ornithonyssus*, *Cheyletiella*, *Pyemotes* and *Dermatophagoides* which may be difficult to identify.

A variety of dermatological disorders, including infectious

diseases of the skin as well as chemical and mechanical irritants, may be experienced or misinterpreted by the patient as a parasite infestation of the skin. During substance intoxication and withdrawal, sensory perceptual disturbances involving the skin may also be experienced. This includes certain medication, cocaine and amphetamine intoxication and the very common sensory hallucinations of alcohol withdrawal delirium. Delusions of parasite infestation of the skin may occur alone or in association with other psychological and psychiatric disorders. It may be experienced in patients with major depression, schizophrenia and delusional disorder (delusional parasitosis). Patients with hypochondriasis are overtly worried about having a physical illness and may misinterpret any symptom of the skin as parasite infestations.

Delusional parasitosis is a chronic psychiatric disorder in which patients have a false and fixed belief that they are infested with parasites. Although it is a psychiatric disorder, patients usually seek help from dermatologists. It is not a mere phobia. The condition is probably more common than generally realised. It occurs in both sexes, but in the older age group women are affected more than men.

The potential of Neem products for control of African ticks

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Ticks and tick-borne diseases are considered one of the greatest animal disease problems in Africa. The conventional method of tick control using chemical acaricides is fraught with several problems such as environmental pollution, chemical residues in meat, milk products and in wool, development of tick resistance and the exorbitant costs. Alternative environmentally friendly and cost-effective methods of tick control are therefore needed. Derivatives of the Neem tree (*Azadirachta indica*) have been traditionally used by farmers in Asia and Africa to control insect pests of households, agricultural and medical importance. Although Neem has been used for centuries for the control of household and agricultural pests, very limited research has been conducted on its potential for controlling African ticks.

The objective of this study was therefore to determine the bioacaricidal efficacy of 4 Neem formulations for the control of 3 major livestock ticks namely; *Rhipicephalus appendiculatus*, *Amblyomma variegatum* and *Boophilus decoloratus* under laboratory and semi-field conditions. The Neem preparations were applied on rabbits and cattle before or after tick attachment by spraying or brushing. In some experiments, ticks were attached to goats after which the animals were allowed to feed on food mixed with Neem seed powder. The test materials at high concentrations *in vitro* induced significant mortality of immature stages of *A. variegatum* and to a lesser degree of adults. Furthermore, the materials when applied to ticks *in situ* caused high mortality of immature stages feeding on rabbits. The mortality was concentration dependent. Neem treatments significantly reduced tick attachment, engorgement, fecundity and hatchability of eggs. Ticks allowed to engorge on goats fed on a diet containing Neem seed powder exhibited a significant reduction in ability to attach and engorge. Feeding and molting periods were also prolonged in *A. variegatum*. Spraying diluted Neem oil on de-ticked Zebu cattle grazing in tick-infested pastures significantly reduced the number of ticks attaching for 4–5 days. These results show that Neem products can provide a suitable environmentally friendly and cost-effective method of managing African ticks.

Strigeoid larval stages in fish of the Okavango Delta, Botswana

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Strigeata is a fairly abundant group of trematodes which comprises strigeoid digeneans with an indirect host life cycle parasitising reptiles, birds and mammals. Larval stages of the families Diplostomatidae and Strigeidae are often found in freshwater snails

and fish. The objectives of this study were to investigate the strigeid cercariae shed by various snail species in the delta as well as to study possible links between snail secretions and strigeoid cysts in fish of the Okavango Delta. Snails were sampled with scoop nets and placed in light for the spontaneous release of cercariae. Fish were caught by means of cast nets. The organs were then examined for parasites. Cercariae and metacercarial stages were studied by light- and scanning electron microscopy employing standard preparation techniques. Six snail species were found to secrete 9 different cercariae, of which only 2 were identified as strigeid cercariae that were secreted by *Lymnaea natalensis* and *Biomphalaria pfeifferi*. *Synodontis vanderwaali* was infected with metacercarial cysts, both in the viscera and on the liver. *Synodontis nigromaculatus* was, however, infected with cysts on the gills, gill arches, skin and in the viscera. *Brycinus lateralis*, *Rhabdalestes maunensis* and *Petrocephalus catostoma* were infected with non-encysted metacercariae in the brain and eyes. *Serranochromis macrocephalus* and *Oreochromis macrochir* on the other hand, had non-encysted metacercariae on the swim bladder tissue. Many more metacercarial cysts are found in other fish species and in order to find possible links in the life cycles of these parasites in the unique wilderness of Africa, experimental infections in fish with different types of cercariae will have to be performed.

Recent *Theileria parva* outbreaks and the risk of re-emergence of cattle theileriosis in South Africa

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The current nomenclature of *Theileria parva* (Bettencourt *et al.* 1907) has not recognised subspecies of *T. parva* reverting to different forms of the disease according to pathogenicity and derivation. East Coast Fever (*Theileria parva* infection in cattle transmitted by the brown ear tick, *Rhipicephalus appendiculatus*) was introduced from East Africa to south of the Zambezi in 1901/02, spreading through most of its vector range. It was subsequently eradicated from South Africa by 1955. Corridor disease (*T. parva* infection in cattle derived from buffalo), transmitted by the ticks, *R. appendiculatus*, *R. zambeziensis* and *R. duttoni* (Angola), was first diagnosed in South Africa in 1955 in the Corridor between the Hluhluwe and Umfolozi Game Reserves in KwaZulu-Natal (KZN). It is usually recognised as a fatal disease in cattle and occurs sporadically in this area wherever there is contact between cattle and carrier buffalo. In 1994 it was stated that 'Corridor-disease has not been reported for many years'. However, since then, trade in buffaloes has escalated accompanied by the introduction of breeding programmes to supply 'disease free buffaloes' to the increasing demands of the tourism industry. A total of 1574 buffaloes were screened for Corridor disease in 2001, 2506 in 2002, 4080 in 2003, and 2351 in 2004 (6 months) for the purpose of translocation. The number of buffalo-associated theileriosis outbreaks in Hluhluwe and Vryheid (State Veterinary Areas in KZN) in 2002–2004 were 8 (75 cattle died in 5 outbreaks) and 6 (79 died), respectively. Theileriosis outbreaks, which had not been associated with buffalo contact, were investigated on 1 farm from 2000–2004. This farm has no borders with any game farm that keep buffaloes, and there is no farm known to have kept buffaloes in the district. Clinical, parasitological, *post-mortem*, serological and molecular diagnoses have all demonstrated the presence of *T. parva* in these outbreaks (34 cattle died). This scenario suggests a re-emergence of a form of *T. parva* which is transmitted between cattle populations and which could have a devastating effect on the cattle industry if not promptly curtailed.

A survey of parasitic helminths in equines from communal farms in the northeastern Free State province in South Africa

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A survey of helminths occurring in horses at Kestell in the north-

eastern region of the Free State province was conducted for a period of 10 months (June 2002 – March 2003). The aim was to determine the factors influencing helminth parasites infections and to record the ticks of veterinary importance in horses in the northeastern Free State. Blood, faecal samples and ticks were collected from 24 horses of the same farms every month for a period of 10 months. The age of horses was between 5 months and 7 years. Collection was done from both males and females. Faecal samples were collected from horses for identification of helminths, blood was collected to perform PCV and ticks were collected for identification and recordings. McMaster, coprological and Visser sieve techniques were used for egg counts. A total of 3 helminth species and a protozoan were recovered. Dominating species were strongyle; with egg per gram (EPG) values ranging between 0 and 4400. An *R* test showed that there was significant difference between age ($P = 0.0218$), seasonality ($P < 0.0001$) and physical condition ($P < 0.0001$) in the prevalence of strongyle. This means that the younger horses had higher infestation levels; colder months showed lower infestation rates and the better the physical condition of the horse the lower the infestation. It also showed that there is a strong linear relationship between packed cell volume (PCV) and ($r = -0.23465$, $P = 0.0004$).

Larval identification was done through preparation of faecal cultures. Small strongyle larvae made up more than 80 % of larval cultures in all samples cultured. Blood samples were collected to conduct a packed cell volume (PCV) test. The readings were found to be normal, ranging between 24 and 44. *Rhipicephalus evertsi evertsi* and *Boophilus microplus* were the only 2 tick species collected from the horses. A questionnaire survey was concurrently carried out to determine the influence of socioeconomic factors on the management of horses, which may favour helminth infection in these species. From the owners interviewed 25.8 % were pensioners and unemployed. A total of 67 % of horse owners utilise their horses' everyday for transport, but the frequency ranged from once to twice a week. Only 39 % of owners use herbal or natural products or drugs to treat their animals, whereas 23 % use commercial products. Horses belonging to 90 % of the owners were given lucern hay all year round, a few owners fed their horses on mealies and other utilised the veld to feed their horses. The disease conditions reported were worms, ticks, eye lesions, hoof problems, skin problems and infectious diseases. Forty-five per cent of owners asked their friends or neighbours who had knowledge of horses for advice when their animals became sick and 42 % treat the animals themselves. Nineteen per cent reported that they did nothing and 6 % took their animals to the state veterinarian. Approximately 3 % made use of animal health inspectors, private veterinarians and traditional healers. Many owners (87 %) allowed their horses to roam free during the day and about 84 % of owners kept their horses in enclosures or small camps during the night.

The information gathered from this study provides the 1st documentation on helminths of veterinary importance in horses in the northeastern Free State.

Larval digenetic trematode parasites in water bodies in the proximity of Pretoria

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Freshwater snails are the 1st intermediate hosts in the life cycles of all parasitic trematodes/flukes. They secrete different types of cercariae that penetrate many vertebrates and invertebrates, ending in metacercarial stages. The objectives of this project were to study the larval forms of digenetic trematodes (cercariae and metacercariae/cysts), to gather more information on their possible life cycles and their role within these water bodies. Field collections were made from different water bodies (*e.g.* farm dams, ponds and rivers). Freshwater snails, fish and freshwater shrimps were collected, identified and examined for larval digenetic parasitic infections where after parasitic stages were examined by light- and scanning electron microscopy.

Four snail species were collected, namely: *Lymnaea natalensis*, *Bulinus tropicus*, *Ferrissia fontinalis* and *Biomphalaria pfeifferi*. The snails secreted a total of 10 different cercarial types. *Lymnaea natalen-*

sis secreted the following cercarial types: xiphidio, echinostome, clinostomatid, 2 strigeid types and the cercariae responsible for *Trichobilharzia*. *Bulinus tropicus* secreted an amphistome cercaria forming the parasite *Calicophoron microbothrium* in cattle, as well as a strigeid cercaria. *Ferrissia fontinalis* secreted a strigeid cercaria, and *B. pfeifferi* a gymnocephalous cercaria. Other larval stages that were collected included the following: strigeid cysts in muscle tissue of *Pseudocrenilabrus philander* and *Clarias gariepinus*; xiphidio cysts in freshwater shrimps, *P. philander* and *L. natalensis* and euclinostome cysts in *P. philander*. There has been the initial difficulty of a lack of descriptions of many cercariae and other larval stages by earlier workers in this field. In many digenean species, only the adult worms have been described morphologically while the larvae remain undescribed. The links between the larval and adult stages of different organisms are unknown in many species and should receive attention.

A survey of coccidian infection (Apicomplexa: Eimeriidae) of freshwater fish in South Africa

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Fish coccidia, especially *Goussia* and *Eimeria* spp., are common and frequent parasites of fish. The majority of the known species has been described from the Eurasian continent. Little is known, however, of the coccidian infections of South African freshwater fish. In September 2003, 77 specimens from 7 freshwater fish species were harvested at 4 sites from rivers and ponds in the Gauteng, North West and Limpopo provinces in South Africa. The fish were surveyed for coccidian infections. Two fish species were infected with apicomplexans belonging to *Goussia*. In banded tilapia (*Tilapia sparrmanii* Smith) unsporulated oocysts of *Goussia vanasi* Landsberg & Paperna, 1987 were found. These sporulated in tap water within 24 hours. Another species in the gut of the chubhead barb (*Barbus anoplus* Weber) harboured sporulated oocysts in the faeces and in the intestinal epithelium. The latter species has been described as *Goussia anopli*. This short survey suggests that more thorough studies might reveal the occurrence of further coccidian species in South African fishes.

Diagnosis of *Theileria equi* in horses belonging to resource-limited farmers in the eastern Free State, South Africa

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Babesia equi, a tick-transmitted protozoan parasite and one of the causative organisms of equine piroplasmiasis, is widespread in South Africa and interferes with the export of horses to disease-free countries such as the United States, Australia and Japan. This work reports on a study that evaluated the usefulness of polymerase chain reaction (PCR) for routine detection of *Babesia equi* in horses. Blood samples from a total of 109 horses, including 7 horses from Kaal plaas, were examined for the presences of *B. equi* using Giemsa-stained blood smears, immunofluorescent antibody test (IFAT) and PCR. Giemsa-stained blood smears and serum samples were immediately prepared from blood samples on arrival at the university laboratory. Aliquots of blood samples from EDTA-coated vacutainers were transferred into marked cryogenic vials and stored at -34°C for further use in PCR. A PCR system that amplifies target of 664 bp regions of the 16S rRNA genes designed and demonstrated specifically to detect the genome of *B. equi*, was used for the purpose of this study. Microscopic examination of Giemsa-stained blood smears from the eastern Free State revealed no parasites compared to serological analysis that showed 98 % of animals possessing antibodies against *B. equi* and 47 % having antibodies against *B. caballi*. PCR was negative for all the 102 horses from the eastern Free State.

A total of 7 blood smears from Kaal plaas revealed that 4 animals were positive for *B. equi* and the other 3 were negative. All 7 animals were found to have antibodies against *B. equi*, but only 3 were *B. caballi* positive compared to results obtained by PCR which successfully produced bands for all the horses.

In vitro investigation of the repellent effects of essential oils of *Tagetes minuta* on *Hyalomma marginatum rufipes* adults

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Due to the numerous problems caused by ticks, tick-borne pathogens and the current methods of control, the demand for alternative tick repellents such as botanicals is sensible and desirable. In the current studies, suitable *in vitro* assays were used to screen *L. javanica* and *T. minuta* essential oils for repellency on *Hyalomma marginatum rufipes* (adults). Two-month-old adults of *H. m. rufipes* ticks were used for this study and essential oils were extracted from *T. minuta* and *L. javanica* using a Clavenger type apparatus followed by identification of chemical components by means of TLC and GC-MS. Three types of climbing repellency bioassays, i.e. A (one-choice bioassay), B (avoidance bioassay) and C (2-choice bioassay) were used. Oils from *T. minuta* and *L. javanica* were found to contain several volatile compounds including monoterpenes. In bioassay A, there was a significant difference ($P < 0.05$) as concentration increased from 0 % to 10.667 % V/V, on transformed data (arc sin square root of proportion repelled for both male and females); *L. javanica* ($df = 3, 23, F_{\text{observed}} = 82.09$ and $F_{\text{critical}} = 3.03$), *T. minuta* ($df = 3, 23, F_{\text{observed}} = 70.21$ and $F_{\text{critical}} = 3.03$). The EC_{50} (repellency) between *T. minuta* and *L. javanica* essential oil, however, was not significantly different. In bioassay B, the proportion of ticks that avoided *L. javanica* essential oil was significant compared to that of *T. minuta* for both male ($P = 0.000037$) and female ($P = 0.000029$) ticks, while in bioassay C there was a significant increase ($P < 0.05$) in repellency with increasing concentration for both *T. minuta* and *L. javanica*. The results from this study suggest that both essential oils of *T. munita* and *L. javanica* repel adult *H. m. rufipes*.

Malaria epidemiology in rice-growing region of northern KwaZulu-Natal in South Africa

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There is a rising concern that rice cultivation increases mosquito populations and thus influences malaria prevalence. This study was undertaken to determine the influence of rice cultivation on malaria prevalence in a malaria-endemic rice-growing area of Mambene in northern KwaZulu-Natal. Mosquito larvae and adult populations were collected on a monthly schedule in 2 rice-growing seasons. Adult mosquitoes were sampled by window exit traps from 17 randomly selected houses where Indoor residual spraying (IRS) was performed. Larvae were sampled using a classical scooping method by a 25 ml ladle at a 2 × 2 m² area. The PCR method was applied to further identify the vector species and blood meals of blood fed mosquitoes also to detect plasmodium parasite gametocytes. The results show that the most abundant mosquito species throughout the larval sampling was the *Culicine* species as compared to the well-known *Anopheles gambiae* that resides in rice paddies in other African countries. The PCR analysis revealed no presence of the plasmodium parasite gametocytes. Furthermore, the *Anopheline* vector mosquitoes collected from the rice paddies did not correlate with the indoor resting population index from the house collections. Although malaria vector densities were high, no malaria death cases were reported within the study area, only 7 clinical cases of malaria were reported. This suggests that the rice cultivation does not contribute much to malaria transmission in the area.

Description of a new actinosporean type from South African freshwaters

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The 1st report on actinospores described these organisms as parasites related to myxosporeans. For a long time this group of parasites was believed to represent an independent taxonomic entity. Research on actinosporeans became more intensive after it was shown that they corresponded to the intraoligochaete developmental stages of fish-parasitic myxosporeans. The relevant research includes earlier surveys, as well as studies of actinospore infection of oligochaetes in natural waters and fish farms in connection with the life cycle of myxosporeans. Relatively little data are available on myxosporean infections of African freshwater fishes and only a single paper appeared in Africa on their actinosporean alternative stages infecting oligochaetes.

In September 2003, actinospore infection of oligochaetes collected from the mud of 2 freshwater biotopes in South Africa was studied. Using the 'cell-well plate method', a new aurantiactinomyxon type was found in 1.1 % of the examined *Branchiura sowerbyi* oligochaete specimens from Rietvlei River northwards to Johannesburg, Gauteng. In 1.5 % of *Branchiura sowerbyi* collected in a pond (Padda Dam), near the Rand Afrikaans University, Johannesburg, the same aurantiactinomyxon type was found. Infected oligochaetes were found only after collection, no actinosporean release was recorded in *Branchiura* specimens kept alive for several weeks. Actinospore infection showed high intensity in oligochaetes in both positive cases. Until now, no actinosporean stages of myxosporeans have been described from South Africa. The aurantiactinospore type presented in this paper differs from the already known types described in the special literature.

Reproductive strategies of branchiurans

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The genus *Argulus* Müller, 1785, has a worldwide distribution with 34 species known from Africa. In southern Africa the only 2 known freshwater species is *A. japonicus* Thiele, 1900, an introduction from the Far East, and *A. capensis* Barnard, 1955, found in the southern Cape. Four species are known from marine and estuarine water around the southern tip of Africa, which includes *A. belones* Van Kampen, 1909, and *A. multipocula* Barnard, 1955, of which female specimens have again been recorded since the original description, but the males are still elusive. This is not uncommon for other known argulids, i.e. *A. angusticeps* Cunningham, 1913, *A. capensis* and *A. fryeri* Rushton-Mellor, 1994. In 2 cases, *A. exiguus* Cunningham, 1913, and *A. gracilis* Rushton-Mellor, 1994, the females are unknown and the descriptions are based only on the males. In the endemic African genus *Chonopeltis* Thiele, 1900, the males of all 14 species have been described. *Dolops ranarum* Stuhlmann, 1891, has a pan-African distribution and males have been described from different populations.

Reproduction in members of the *Branchiura* involves the transfer of sperm to a spermathecae in the case of *Argulus* and *Chonopeltis* and the transfer of a spermatophore in the case of *Dolops* Audouin, 1837. Are males still needed after sperm transfer? In order to elucidate the reproductive strategy, the reproductive biology of these branchiurans is compared.

Burden of malaria in pregnancy in KwaZulu-Natal: preliminary results

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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 the Intermittent Preventive Treatment for all pregnant women at risk of malaria. This study was initiated to determine the burden of malaria in pregnant women. This information is required to support implementation of Intermittent Preventive Treatment policy in South Africa. Data collection commenced in May 2004 in 3 health facilities in Umkhanyakude district, KwaZulu-Natal. Data include demographic characteristics, routine antenatal care attendance, history of malaria during pregnancy and use of insecticide-treated bednets. In total, 323 women attending antenatal care for the 1st time have been recruited. Of these women, 30 % are teenagers and have primary or no education. Almost all of the women are single and unemployed. Twenty-two per cent of these women own insecticide-treated bednets.

The levels of both anaemia and HIV are unacceptably high, but the prevalence of malaria is very low in these women (4.3 %). The high level of anaemia could be attributed to HIV or other factors since the prevalence of malaria is low. The low prevalence of malaria is expected since the study is ongoing and only a third of the expected sample has been obtained. Another reason could be the fact that data collection commenced after the malaria transmission season. Data presented here are preliminary and therefore conclusions regarding the burden of malaria in pregnancy cannot be made until the study is complete.

Aspects of the pathology of *Lamproglena clariae* from the Vaal River system, South Africa

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Lamproglena clariae Fryer, 1956, attaches to gill filaments of *Clarias gariepinus*, a widely distributed fish in Africa. It penetrates the gill tissue and consumes blood; hence the purpose of this study was to determine the effect of its attachment and feeding on the gill filaments of the host. Fish were killed by a single cut through the spinal cord and gills were dissected. They were studied under the light microscope and gill filaments with adult females *in situ*, were fixed in an alcohol, formaldehyde and acetic acid solution and then preserved in 70 % ethanol. Specimens were stained with Azocarmine-Aniline Blue and studied microscopically. An inflammatory reaction in the form of swelling in the vicinity of the young adult and a capsule that was formed around the embedded head of the adult *L. clariae* were observed on the gill filaments. Histological examination revealed fusion of gill lamellae and erosion of tissue at the attachment zone. Hyperplasia of the goblet cells was also revealed. The only affected filaments were the ones with attached parasites while the rest remained normal. Although these results show that *L. clariae* caused localised infestation, the high intensity of infestation may be detrimental to the fish. Hyperplasia of goblet cells can cause respiratory distress and suffocation of the fish. Fusion of lamellae would result in restriction of air passages and thus hinder the respiration of the host.

Some aspects of the biology of the monogenean *Dendromonocotyle colorni* from the skin of stingrays from Sea World at Ushaka Marine World

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The monocotylid genus *Dendromonocotyle* comprises 11 species, which have all been recorded from the skin of stingray species representative of the families Dasyatidae, Myliobatidae and Urolophidae. Globally there are increasing reports of *Dendromonocotyle* infestations of stingrays particularly in public aquaria. *Dendromonocotyle colorni* has recently been recorded from the sharpnose stingray (*Himantura gerrardi*), round ribbon tail ray (*Taeniura melanospilos*) and the honeycomb stingray (*Himantura uarnak*) at Ushaka Marine World, Durban. Adult *Dendromonocotyle*

colorni specimens were removed from the infested hosts and allowed to lay eggs. Adult worms started producing straw-coloured tetrahedral eggs immediately after removal from the host. Eggs were embryonated in filtered seawater at 17, 23, 26 and 30°C. Larval development and embryonation periods were recorded daily at each temperature. Initial development was evident on day 3 across the temperature ranges and could be seen in the development of larval eyespots and hooklets. Temperature appeared to affect further development and eggs hatched after 6–12 days across the temperature range. Hatching was not spontaneous but required specific stimuli, or larvae would die in the egg after days 10–12. Further investigations into the biology and life cycle of *Dendromonocotyle colorni* may provide opportunities to explore integrated parasite management strategies which are more suited to treating these parasites in public aquaria.

Posters

Changes in the prevalence and intensity of intestinal protozoan infections in children after receiving anthelmintic treatment

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Intestinal helminths usually co-exist with intestinal protozoans in infected individuals, and although the helminths have successfully been brought under control by chemotherapy, very little has been done to determine the effect of this treatment on the protozoans. In order to determine the effect of anthelmintic treatment on concomitant protozoan infections, the prevalence and intensity of the common intestinal protozoans and helminths in primary school children were determined before and after helminth chemotherapy. Four surveys were done and these involved the collection and examination of stool samples using the formalin-ether concentration method. There were significant decreases in the prevalence and intensity of *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm (presumably *Necator americanus*) after treatment, while the prevalence and intensity of *Entamoeba histolytica*, *E.coli*, *E. hartmanni*, *Endolimax nana*, *Iodamoeba butschlii*, *Giardia intestinalis* and *Chilomastix mesnilli* increased significantly. It is recommended that individuals who have been treated for nematode infections should also be examined for protozoan infections so that these are treated accordingly.

Micromorphological specializations of the falcon louse *Laemobothrion tinnunculi*

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Laemobothrion tinnunculi is a very large louse that is an obligate ectoparasites infesting the feathers of several falcon species, many of which are endangered. These birds are also used in falconry and the lice used in this study were collected from a Lanner falcon at a veterinary clinic in Dubai. Apart from their taxonomical characteristics, little is known about these lice. This study was aimed at investigating the micromorphology of *L. tinnunculi* by scanning electron microscopy (SEM). The lice were fixed in 70 % ethanol. After ultrasonic cleaning, the lice were routinely prepared for SEM and viewed in a Leica Stereoscan 420 scanning electron microscope at 5 to 10 kV.

The study revealed a number of specializations of these lice that were very dorsoventrally flattened to fit between the feather barbs. The mandibles were specialised to grasp and shear off pieces of the feather barbule. The anterior maxillary palpi had 4 segments with the distal tip bearing 14 peglike terminal sensilla which were arranged differently from those in *L. maximum*. The medial labial palpi had 5 terminal, grooved sensillae which is characteristic of *Laemobothrion* species. The antennae were located in antennal pits which resembled the antennal fossae in fleas. Posterior to the antennal fossae were the eyes, each consisting of a pair of separate

lens-covered ommatidia. Ventral to each eye were the cephalic ctenidia, forming a unique organ of unknown function, which consisted of 6 comb-like rows of setae adjacent to an area covered by small scales. This organ may be of taxonomic importance as only 5 rows of setae have been reported in both *L. maximum* and *L. vultures*. The separate prothorax was well developed while the fused meso-metathorax resembled the abdominal segments in shape. Each of the legs terminated in 2 horn-shaped tarsal claws without the normal opposing pretarsal setae found in other feather infesting lice. The femur had a medial groove ventrally, into which the tibia fitted, enabling the feather barbules to be held firmly, as described in *L. vultures*. The lateral area of femur III as well as the lateral areas of sternites IV were covered with microtrichia arranged as short comb-like structures, which are characteristic of the sternites IV–V of other *Laemobothrion* species. However, the number of microtrichia per comb has been reported as varying between 2 to 7, but in *L. tinnunculi* it was found to be between 3 and ten. The round abdominal spiracles were carried dorsally on the tergites of the abdominal segments III–VIII, while segments I–II only had small stigmatal scars. A pair of large, comma-shaped thoracic spiracles was seen on the lateral surfaces of the prothorax. The lumens were lined by parallel lamellae to form a fine air filter.

Parasites of *Oreochromis mossambicus* as bio-indicators of water quality

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In recent years, an increasing number of papers have focused on parasites as useful indicators of pollution. Pollution can 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 a particular pollutant than the host, or pollution levels eliminate the suitable intermediate host. The parasites from the Mozambique tilapia formed part of a research project on the assessment of fish health and water quality in the polluted lower reach of the Ga-Selati River (Olifants River System) of the Limpopo Province. Ecto- and endoparasites were collected seasonally over a period of 2 years from 2 polluted sites and 1 unpolluted site near the river's confluence with the Olifants River at Phalaborwa. Parasites were fixed and preserved using standard methods. A Parasite Index (PI) was determined. The results indicate that the water quality is poor at the sampling sites of the mines caused by the non-toxic constituents magnesium, chlorides and sulphates, while metal concentrations were acceptable. The sampling site upstream from the mines showed lower TDS values. The following parasites were recorded: monogeneans from the gills (*Cichlidogyrus* spp.); digeneans from the eyes, gills, swimbladder (*Diplostomum* larvae) and skin ('Black spot'); cestodes from the liver (dilepidid larvae); nematode larvae from the body cavity (*Contracaecum* sp.); pentastomid larvae from the swimbladder (infective stage); branchiurans (*Dolops* sp. and *Argulus* sp.) from the skin and copepods (*Lernaea cyprinacea*) from the skin. The hypothesis that the number of ectoparasites will be lower in polluted water and that of endoparasites will be higher was not supported for this host. However, the PI has potential and should be tested in more water bodies of South Africa.

Some parasitic mites of phytophagous insects from western Iran

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Some mites have 2 roles in biological control; in larval stages they are parasitic, while playing a predatory role in their post-larval stages on variety insects, including important phytophagous insects. The effect is a decrease in the populations of phytophagous insects. Parasitic mites were collected and fixed in 70 % ethanol.

Mites were mounted on slides in a modified Hoyer's medium and studied with an Olympus® phase contrast microscope and sketches were made using a drawing tube. In this study 8 species were collected and identified. All species were new recordings for western Iran. Parasitic mites according to their hosts were as follows:

1. Aphids (*Macrosiphom rosae*): *Allothrombium shirazicum* Zhang; *A. pulvinum* Ewing
2. *Aphis sophorae*: *Erythraeus (Zaracarus) logipedus* Saboori & Nowzari
3. Unknown aphid: *Erthraeus (Zaracarus) sp.*; *Erthraeus (Zaracarus) iranicus* Saboori & Akrami
4. Walnut aphid: *Erythraeus sp.*
5. Grasshopper: *Leptus sp.*
6. Sunn pest (*Eurygaster integriceps* Puton): *Leptus esmailii* Saboori & Ostovan
7. *Erythraeus (Zaracarus) kurdistanensis* Khanjani & Ueckermann
8. *Hypoaspis (Hypoaspis) polyphyllae* Khanjani & Ueckermann

Parasite diversity of *Pseudocrenilabrus philander* in farm dams near Pretoria

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Pseudocrenilabrus philander (the Southern Mouthbrooder) is commonly found in many water bodies in South Africa, and although they are relatively small in size they provide a niche for many different types of parasites.

Fish were sampled from 2 farm dams near Pretoria by using hand nets. Examination of fish included skin and gill smears, as well as muscle tissue and visceral tissue scans for the presence of parasites. Ciliated protozoa were stained with haematoxylin and impregnated with silver nitrate, while other parasites were studied by light microscopy and scanning electron microscopy utilising standard techniques.

The following parasites were found: 1) on the skin, 2 Mobilina ciliates, i.e. *Trichodina heterodontata* and *T. centrostrigata* and 2 Sessilina ciliates which included an *Epystilis sp.* and an *Apiosoma sp.*; other infestations of a monogenean *Gyrodactylus*, as well as a myxosporean of the genus *Myxobolus* were found in cysts; 2) the gills on the other hand, provided a habitat for *T. centrostrigata* and a monogenean *Dactylogyryus sp.* and further investigation of the viscera revealed an encysted larval roundworm infection on the organs; 3) additionally, many encysted metacercariae of the strigeid type were found on both the skin and gills of infected fish.

Pseudocrenilabrus philander can act as an ideal model to illustrate parasite diversity since many sessilina ciliates are often found on the skin of these fish. The *Myxobolus sp.* found in this study, however, seems different from the material collected from other localities. The numerous metacercarial cysts that were observed also indicate that freshwater snails secreting strigeid cercariae were present in the same water body.

Parasites of *Clarias gariepinus* as bio-indicators of pollution

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Parasites interact with environmental pollution in different ways. Parasites can interfere with established bio-indication procedures owing to their effect on the physiology and behavior of the host. This could lead both to false-positive and false-negative indications of pollution. Parasites can also be used as bioaccumulation and effect indicators because of the variety of ways in which they respond to pollution. The Ga-Selati River was selected for this study due to the industrial and mining activities on its banks before its confluence with the Olifants River. Two sampling sites in the vicinity of the mines and 1 site representing an unpolluted part of the river were sampled seasonally. Water quality and the abundance, intensity and prevalence of parasites were determined to calculate a Parasite Index (PI). Parasites were fixed and preserved using standard methods. The following parasites were recorded: protozoans from the blood (*Trypanosoma sp.*); monogeneans from the gills (*Quadriacanthus sp.*; *Macrogryrodactylus sp.*) and skin (*Macrogryrodactylus sp.*);

digeneans from the gills and muscle (larval forms); adult digeneans from the hindgut (*Glossidium sp.*); cestodes from the intestine (*Polyonchobothrium sp.* and *Bothriocephalus sp.*); nematode larvae from the body cavity (*Contracaecum sp.*); adult nematodes from the stomach and hindgut (*Paracamallanus sp.*); leeches from the skin; branchiurans (*Dolops sp.* and *Argulus sp.*) from the skin and copepods from the gills (*Lamproglena clariae*). The hypothesis that the number of ectoparasites will be lower in polluted water and the number of endoparasites will be higher was well supported at the mine sites where a higher endoparasite index was recorded. The non-impacted site, however, did not support the hypothesis. It is clear that increases and decreases of parasite numbers depend largely on the type of pollution and more research is needed to establish whether parasites will be valuable in environmental pollution.

Detailed morphology of the copepod parasite *Nesippus orientalis* (Siphonostomatoida, Pandaridae)

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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 its host. Morphologically it belongs to the *Dinemoura*-group of the Pandaridae, which is distinguished from the *Pandarus*-group by the 2nd free thoracic segments without dorsal plates. A revision of the family Pandaridae was done in 1967 during which *N. orientalis* was redescribed. This description lacked finer detail about the armature of appendages. Since a number of previously new species turned out to be synonymous with *N. orientalis*, detailed descriptions and illustrations may prevent this confusion in future.

Nesippus orientalis specimens were collected from different elasmobranch hosts at the facilities of the Natal Sharks Board, Umhlanga Rocks, KwaZulu-Natal. Collected material was fixed and preserved in 70 % ethanol. Studied specimens were cleared in lactic acid into which a little bit of lignin pink was dissolved before being dissected and examined using the wooden slide technique. Drawings were made with the aid of a drawing tube. Selected specimens were also prepared for scanning electron microscopy using standard methods. Drawings and electron micrographs are used to illustrate detail on the fine structure of *N. orientalis*. Details of the armature of appendages varied in previous descriptions as well as in the current study, but this is the 1st report on the presence of fingerlike structures, posterolateral to the mouth tube, which have never been reported before.

Livestock diseases in communal grazing areas of the north eastern Free State, South Africa

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This is a summary of a 5-year study conducted by the University of the Free State – Qwaqwa Campus Parasitology Research Programme in 3 study sites at Harrismith, Kestell and Qwaqwa in the northeastern Free State province of South Africa from 1998–2003. A variety of tick species of economic importance are found in the northeastern Free State. The following have been identified from cattle: *Boophilus decoloratus*, *Rhipicephalus evertsi evertsi*, *R. foliis*, *R. gertrudae* and *R. punctatus*. Most of the common infectious diseases as well as poor animal body condition are frequent and include alopecia, rumen alkalosis, abscesses, traumatic reticulo-pericarditis, anaplasmosis, keratitis, pneumonia, lumpy skin disease, ephemeral fever and stomatitis. Approximately 94 % of cattle are seropositive for *Babesia bigemina* by IFAT while 87 % are seropositive for *Anaplasma* by ELISA. The high incidence of positive *Babesia* and *Anaplasma* by serology, but negative smear results for the same

sample of animals indicates a stable disease situation. Tick species that infect sheep and goats have been identified as *Rhipicephalus evertsi evertsi* and *Boophilus decoloratus*. All sampled sheep and goats were seropositive for *Theileria* by IFAT while 85 % of sheep and 100 % of goats were seropositive for *Anaplasma*, respectively, by competition inhibition ELISA. Cattle generally had low helminth infections, with egg per gram values of between 0 and 400 while goats and sheep had relatively high values varying from 20–2550. Dominant species infecting cattle were *Haemonchus* (EPG 0–125) and *Oesophagostomum* (EPG 0–120) while in small stock dominant species were found to be *Haemonchus* (EPG 20–2550) and *Bunostomum* (EPG 0–1340). *Eimeria* species (gastrointestinal protozoan) was isolated at high EPG values (EPG 1–1100) from cattle, goats and sheep. In horses, the following 3 helminth species were identified: Strongyle, *Parascaris equorum* and *Oxyuris equi*, with the predominant species being strongyle (EPG 0–4400). *Eimeria* was isolated at low EPG values of 0–100. Approximately 5 % of free ranging chicken have been exposed to Newcastle disease, 43 % to infectious bronchitis and 63 % to *Mycoplasma gallisepticum* infection. Helminths isolated at low EPG values included *Heterakis*, *Ascaridia* and *Capillaria* species. *Eimeria* species were also isolated. The red fowl mite *Dermanyssus gallinae* was isolated from some of the birds and from their nests. Results from these studies will form an important basis from which appropriate control programmes for diseases in live-stock can be formulated, particularly for emerging financially constrained farmers.

Monogenean parasites of 5 fish species in the Okavango River, Botswana

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Monogeneans are ectoparasites of both marine and freshwater fish and are highly host- and site specific. They are often (but not exclusively) found on the gills and skin of fish where they attach by means of a unique organ. The opisthaptor, is situated at the posterior end of the body and is responsible for the destruction of epithelial tissue around the attachment site resulting in erosion and degeneration of the site. The objectives of this study were to investigate the morphology of the various parasites and to classify them taxonomically. Field collections of different freshwater fish species were made from different localities in the Okavango River, Botswana. Fish species were initially identified and examined for gill monogenean parasites. Thereafter, live monogenean parasites were studied by light microscopy and photographed using a Nikon[®] Coolpix[™] camera.

The monogenea of the following fish species were reported on: *Synodontis nigromaculatus*, *Tilapia rendalli*, *Sargochromis carlottae*, *Serranochromis robustus* and *S. altus*. These fish spp. were infected with different monogenean parasites each equipped with an opisthaptor that bear different combinations of clamps, bars and marginal hooklets. Monogeneans collected from *S. altus*, *S. robustus*, *T. rendalli* and *S. carlottae* are of the genus *Cichlidogyrus* Paperna, 1960 and monogeneans of *S. nigromaculatus* of the genus *Ancyrocephalus* Paperna and Thurston, 1968. Various monogenean genera from the Okavango River were found to be host specific. Monogenea have received little attention in the past (particularly in Africa) and this is the 1st report from these fish species from the Okavango river system. Further investigation directed towards the identification and morphology of these monogenean parasites is therefore essential.

Cercariae shed by freshwater snails around Pretoria, Gauteng

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Freshwater snails are known to serve as 1st intermediate hosts in the life cycles of all parasitic trematodes/flukes, e.g. *Bulinus africanus* serves as host for *Schistosoma haematobium* and *Biomphalaria pfeifferi*

serves as host for *S. mansoni*. They secrete different types of cercariae that penetrate many vertebrates and invertebrates. The objectives of this project were to study the larval forms of digenetic trematodes (cercariae) and to gather more information on their possible life cycles and the role that they play within these water bodies. Field collections were made from different water bodies (e.g. farm dams, ponds and rivers) around Pretoria. Freshwater snails were collected, identified and examined for cercariae. Cercariae were studied by light microscopy using vital stains and scanning electron microscopy employing standard techniques.

Four species of snails were sampled, namely *Lymnaea natalensis*, *Bulinus tropicus*, *Ferrissia fontinalis* and *Biomphalaria pfeifferi*. These snails secreted a total of 10 different forms of cercariae. *Lymnaea natalensis* secreted the following cercarial types: xiphidio; echinostome; clinostomatid; 2 strigeid types, as well as the cercariae responsible for *Trichobilharzia*. *Bulinus tropicus* secreted an amphistome cercaria that matures into the parasite *Calicophoron microbothrium* in cattle, and a strigeid cercaria. *Ferrissia fontinalis* secreted a strigeid cercaria, and *B. pfeifferi* secreted a gymnocephalous cercaria. Of the 10 cercariae shed by 4 different snail species in this area only 1 has been documented, i.e. the amphistome cercaria while the rest of the cercariae are unfamiliar and their life cycles are unknown. It therefore holds a challenge for parasitologists to study such an unknown field in order to solve some of these unique trematode life cycles sharing the space in which we live.

Experimental immunization of rabbits against *Amblyomma hebraeum* using salivary gland lysates and whole tick extracts

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Hosts acquire resistance to ticks either after natural and repeated infestation or after immunization with tick-derived antigens. The effect of experimental immunization of rabbits against *Amblyomma hebraeum* using its salivary glands and whole tick antigens was determined. Partially engorged *A. hebraeum* females were dissected and tick extracts were collected, homogenised and used as crude antigens. Rabbits were immunised subcutaneously with whole tick and salivary gland extracts, followed by 2-booster immunizations after 2 weeks. Experimental and control animals were challenged with 10 adults of *A. hebraeum* (5 males + 5 females) ticks. The study showed that rabbits immunised with whole tick extracts and salivary gland lysates acquired resistance to *A. hebraeum* adult ticks. Reduced number of engorged ticks, reduction in the weights of engorged ticks, prolongation of feeding time and reduction in tick fecundity expressed this immunity or resistance to ticks. Salivary gland antigens were found to be more immunogenic than whole tick antigens. The immunization dose of 3.0 mg had a significant effect on the number of dead ticks (P -value = 0.022). Most of the engorged female ticks collected from immunised animals changed their normal grey colour to reddish-black and died prior to oviposition.

Whole tick and salivary gland proteins were then separated by SDS-PAGE. Several proteins ranging from 38–207 kDa were found to be constituents of whole tick antigens. Three polypeptides with molecular weights of 55, 68 and 80 kDa were found to be constituents of salivary gland antigens. Whole tick and salivary glands homogenates were analysed by Western blotting. Two bands of molecular weights of 68 and 80 kDa from whole tick extracts and an 80 kDa band from salivary gland lysates were recognised by polyclonal immune sera, respectively. Results from this study will provide the basis for the development of a candidate vaccine against *A. hebraeum* in South Africa.

The protozoan blood parasites of the Nile crocodile, *Crocodylus niloticus*, from the Okavango Delta, Botswana

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During routine blood sampling of Nile crocodiles, *Crocodylus*

niloticus, in the Okavango Delta, Botswana, a heavy infection with an unknown protozoan blood parasite was detected in more than 50 % of the crocodiles captured. The presence of protozoans in blood of Nile crocodiles was noted as long ago as 1907, from hosts caught in the Gambia and the Democratic Republic of Congo. Since these initial observations, 2 species of haemogregarines have been described from crocodiles in Africa. The 1st was *Hepatozoon pettiti* (Thiroux, 1910) from crocodiles in Senegal and Uganda and then *Hepatozoon sheppardi* (Santos Dias, 1952) from crocodiles in Moçambique. The Okavango haemogregarine shows morphological and morphometrical similarities with both of these described species. Digital analysis confirms these similarities and final results may indicate that *H. sheppardi* is a junior synonym of *H. pettiti*.

However, although similar in size range and staining properties to the known species, the Okavango parasite differs in infection levels (much higher than reported from Uganda) and also in the effect on the host erythrocyte. The Okavango haemogregarine modifies the shape of the infected erythrocyte from oval to round and also displaces the nucleus of the red cell from the centre to the edge of the cell. These effects were not noted in infections of either *H. pettiti* or *H. sheppardi*. It is thus concluded that although similarities exist between the Okavango haemogregarine and the 2 known species, the taxonomical status of this parasite will be finalised only after research on its vector has been undertaken. This is the 1st record of a blood protozoan infection in crocodiles from the Okavango Delta in Botswana.