

Parasitological Society of Southern Africa

The following are abstracts of papers and posters presented at the 35th Annual Congress of the Parasitological Society of Southern Africa (PARSA), 24–26 September 2006, Ministry of Mines and Energy, Windhoek, Namibia

Keynote Addresses

Malaria in South Africa: A success story

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Malaria is the most important parasitic disease in Africa with regard to morbidity and mortality. Challenges to malaria control include ongoing parasite drug resistance to chemotherapeutic agents and vector resistance to insecticides.

In South Africa, malaria transmission is restricted to 3 provinces. *Plasmodium falciparum* is the predominant species accounting for at least 95 % of cases and all malaria-related mortality. Furthermore chloroquine resistance is widespread.

Malaria case notification rates have decreased from 64 622 cases in 2000 to 13 399 cases in 2004 and 7755 in 2005, largely as a result of a number of initiatives. These include intensive indoor residual spraying programmes, including the use of DDT to combat insecticide resistant *Anopheles funestus*, the introduction of Artemisinin Combination Therapy (ACT) for the treatment of uncomplicated malaria and the Lebombo Spatial Development Initiative, a cross-border collaboration, targeting malaria control in eastern Swaziland, southern Mozambique and northern KwaZulu-Natal.

As well as being highly effective and improving cure rates, ACT has the advantage of diminishing the chances of further parasite drug resistance to sequential monotherapy. The artemisinins also decrease gametocyte development and hence potentially reduce malaria transmission. Diagnosis using highly sensitive and specific rapid malaria antigen tests has facilitated point of care testing in rural clinics. Programmes to monitor drug resistance *in vivo* are ongoing and are essential to monitor the development of resistance and guide further treatment decisions.

Snail-transmitted trematode parasites

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Trematode parasites have a life cycle with potentially 3 hosts. Freshwater snails serve as 1st intermediate host and produce cercariae that penetrate the 2nd intermediate host to form metacercariae. Adults only develop once these infected hosts have been eaten by suitable final hosts. In a few cases cercariae penetrate the final host directly. In order to study the trematode parasites of Gauteng and the Free State, water bodies were surveyed for freshwater snails, which were placed in glass containers for natural shedding of cercariae, and thereafter studied by light and scanning electron microscopy. Other organisms were simultaneously collected during the study and examined for metacercariae. In the Free State the snail *Bulinus tropicus* was found to be the most abundant, whereas *Lymnaea natalensis* and *B. tropicus* were found in most ponds in Gauteng. A variety of cercariae were produced: apharyngeal-brevifurcate-distome forktail cercariae on the one hand, formed *Schistosoma mattheei*, as well as a *Trichobilharzia* sp., in experimentally infected rats, whereas apharyngeal-brevifurcate-monostome forktail cercariae resulted in *Clinostomum tilapiae* metacercariae in a few *Tilapia* fish. The pharyngeal-longifurcate-distome cercariae (mostly single tailed) on the other hand, penetrated freshwater fish where these encysted. *Bulinus tropicus* secreted amphistome cercariae of *Calicophoron microbothrium* that encysted on plants and were eaten by cattle and sheep. Echinostome cercariae with 27-spines (*Petasisger variospinosus*) were found to encyst in tadpoles of *Xenopus laevis* whereafter the experimental life cycle was completed in the reed cormorant, *Phalacrocorax africanus*. Another echinostome cercaria with 43-spines was also encysted in the same

snail species. The life cycle of the latter was experimentally completed in rats and thus identified as *Echinoparyphium elegans*. Xiphidio cercariae were often secreted by other snail species and their life cycles are still unknown, but snails, fish and freshwater shrimps were found to harbour xiphidio cysts. In a changing environment, this field still holds many challenges in future to solve the life cycles of many known and unknown trematode parasites.

Vaccination against *Taenia solium* infection in pigs: a new method for control of cysticercosis

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Neurocysticercosis is a parasitic disease caused by infection with the larval stage of the cestode *Taenia solium*. The disease is an important cause of human morbidity and mortality in many non-Islamic parts of the developing world. Despite the availability of effective anthelmintics, the disease remains prevalent and there is a need for new and improved measures for control of the infection. The parasite is transmitted zoonotically, with pigs acting as the natural animal host. We have sought to develop an effective practical vaccine to prevent infection in pigs as a new option to assist with *T. solium* control. Previous research in our laboratory has demonstrated the potential for use of recombinant oncosphere antigens to be used as vaccines to prevent cysticercosis caused by *Taenia ovis* and *Taenia saginata* in sheep and cattle, respectively, as well as for prevention of hydatid disease caused by infection with *Echinococcus granulosus*. This success has encouraged the adoption of a similar strategy for *T. solium*. Two oncosphere proteins were cloned from mRNA obtained from *T. solium* oncospheres and expressed in *Escherichia coli* as GST fusion proteins. To date, 5 vaccination trials have been undertaken in pigs against an experimental challenge infection with *T. solium* eggs. These trials were undertaken by collaborating scientists in Mexico (two trials), Peru, Honduras and Cameroon. In every trial a vaccine, comprising the recombinant protein TSOL18, induced complete or near complete protection against infection with the parasite (individual experiments: 100 %, 100 %, 99.9 %, 99.9 % and 99.5 % protection). Current research is assessing the duration of protection that is afforded by the vaccine prior to undertaking small-scale field trials which are scheduled for endemic regions in 3 countries. The ultimate objective of this research is for these recombinant oncosphere vaccines to play a major role in the global eradication of neurocysticercosis.

Tsetse-transmitted trypanosomosis in southern Africa: new insights – new challenges

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Tsetse flies occur over large parts of southern Africa and are a considerable threat to sustainable livestock production. However, the impact of the disease (Nagana) on production depends largely on the epidemiological situation in a particular area. In southern Africa, 4 epidemiological situations can be distinguished that have repercussions for the impact and control of the disease in livestock. The epidemiological circumstances can vary in space and in time and are the consequence of changes in the distribution and density of people and their livestock and concomitant changes in the distribution and density of tsetse flies and tsetse host availability. The pressure for land for agriculture (e.g. Malawi, South Africa), resettlement schemes (e.g. Mozambique, Zimbabwe) or restocking programmes (e.g. Mozambique) have especially important repercussions on the epidemiology of Nagana. The impact of the disease

on livestock production is highest where tsetse flies are highly dependent on game animals for their survival and where game animals constitute an important reservoir of trypanosomes. Despite high prevalence in livestock, the impact of Nagana is much lower in areas where cattle have become the main host of tsetse and constitute the main trypanosome reservoir. Maintaining or creating such a trypanosomosis endemic situation may be another tool in the trypanosomosis control strategies. If tsetse control is considered an option, epidemiological circumstances will have to be considered when determining the most appropriate control strategy.

From discovery to development: current industry perspectives for the development of novel methods of helminth control in livestock

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Despite the extraordinary success in the development of anthelmintics in the latter part of the last century, helminth parasites continue to pose the greatest infectious disease problem in grazing livestock systems worldwide. Newly emerged threats to continuing successful livestock production are the failure of this chemotherapeutic arsenal due to the widespread development of anthelmintic resistance at a time when the likelihood of new products becoming commercially available seems more remote. Changing public attitudes with regard to animal welfare, food preferences and safety will also significantly impact on the ways in which livestock are managed and their parasites are controlled. Superimposed on this are changes in international livestock demographics, in response to evolving trade policies and demands for livestock products. Adding to this is the ever-diminishing numbers of veterinary parasitology researchers in both the public and private sector.

Industries, whether they are livestock industries, public research industries or pharmaceutical industries, must adapt to these changes. In the context of helminth control in ruminant livestock, the mind-set of 'suppression' needs to be replaced by 'management' of parasites to maintain long-term profitable livestock production. Existing effective chemical groups need to be carefully husbanded and non-chemotherapeutic methods of parasite control need to be further researched and adopted, if and when they become commercially available. This will require veterinary parasitology researchers from both the public and private sectors to work in close cooperation to ensure 'sustainability' – not only of the livestock industries that they serve – but also for their own activities and enterprises.

Oral Presentations

Comments on the distribution and phylogeny of the Branchiura

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The Branchiura consists of only 4 genera. *Dolops* occurs in South America, Africa and Tasmania. These were connected in Gondwanaland. *Dipteropeltis* is endemic to South America and *Chonopeltis* to Africa. *Argulus* has a cosmopolitan occurrence implying that *Dolops* evolved before Gondwanaland separated and gave rise to *Dipteropeltis* and *Chonopeltis* after the formation of South America and Africa. If *Argulus* is the sister group to *Dolops*, it probably also came into existence during that period and developed the ability to survive in marine and brackish environments which allowed a cosmopolitan distribution. The ontogeny of the maxillulae also suggests that *Argulus* is a descendant of *Dolops*. Furthermore, in *Chonopeltis lisikili* egg-bearing females have a remnant of the hook attached to the sucker in the 1st maxillae, implying a neotenic structure.

As far as host specificity is concerned, *Dolops* is loosely specific to tilapia-like fishes (and silurids), *Chonopeltis* to cyprinids, *Dipteropeltis* uncertain (information limited), but *Argulus* is an opportunist even

within 1 *Argulus* species as observed in *Argulus japonicus*. Species specificity is a characteristic that evolved over generations and involves the development of specific proteins indicating that *Argulus* is a recent addition to the Branchiura.

Pre-oral structures occur only in *Argulus* and *Dipteropeltis*, implying a closer relationship between these 2 genera.

Effect of water levels on parasite infestations in the Okavango Delta, Botswana

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The Okavango Delta is a dynamic ecosystem that is driven by an annual flood, which takes approximately 6 months to flow the distance of around 250 km as the crow flies from Mohembo (at the Namibian border) to the town of Maun in the south. During this extended flood, water starts rising gradually from the end of December at Mohembo, reaching the Gumare fault around March to April and Maun by the end of July. This gradual flood fills the floodplains and in the vanguard small fishes are already present, feeding on insects caught by the rising water. During these winter months, water temperatures fluctuate, dropping at night and rising during the sunny, warm winter days. These fluctuations, as well as crowding of fish in the relatively shallow water, stress the fish populations. One of the consequences of this stressed condition is a weakened immune system coupled with extremely high loads of parasites, specifically ectoparasites. During conditions like these the floodplains in the delta are vast pools of extremely high parasite biodiversity, with normal over-dispersion of parasites completely skewed.

As the floodplains start receding in September, the floodplain pool fish move into the main streams, backwaters and to a lesser extent the lagoons. Fish that remained in these latter habitats the whole time are now exposed to an impressive array of ectoparasites present on the erstwhile floodplain fish. This, and the ever present threatening low oxygen levels in the Okavango system, results in fish that overall seem to harbour a higher level of parasites than would be expected in a natural system.

Evidence of a novel *Babesia* parasite in a domestic cat in South Africa

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Blood parasites of the genus *Babesia* occur in many different hosts of the family Felidae. The 2 most frequently reported species are *Babesia felis*, which causes clinical babesiosis in domestic cats, and *Babesia leo*, primarily reported from asymptomatic lions. A 2-year-old male cat died of suspected babesiosis. Blood smears showed a large *Babesia* parasite. Samples were submitted for nucleic acid-based analysis. DNA was extracted from frozen organs. PCR was performed using primers that amplified a variable region of the 18S rRNA of the parasite. The amplicons obtained were analysed with the reverse line blot (RLB) assay. The samples tested positive for a *Babesia* parasite but did not react with any species-specific probe. The PCR products were sequenced. On phylogenetic analysis, these sequences did not group with *B. felis*, *B. leo* or other small blood parasites such as *B. microti*, but were in a group of their own. These results revealed that a further *Babesia* species may be present in domestic cats.

The spatial distribution of *Nemesis lamna* Risso, 1826, on the gills of the great white shark, *Carcharodon carcharias* (Linnaeus, 1758)

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Nemesis lamna is a parasitic siphonostomatoid occurring on the gill

filaments of great white (*Carcharodon carcharias*), shortfin mako (*Isurus oxyrinchus*) and basking (*Cetorhinus maximus*) sharks. *Nemesis* species can be divided into 2 groups by the relative widths of their cephalothoraces, free thoracic segments and genital segments. One group (consisting of most of the species) has a 4th free thoracic segment much narrower than the preceding 3, while the other (consisting of *N. lamna*) has all 4 segments of about the same width. One complete set of gills of each of 11 great white sharks caught in the nets of the Natal Sharks Board was investigated for infection by *N. lamna*. In order to investigate and analyse the spatial distribution of the copepods, the relationships between host size and gill surface area and gill surface area and number of copepods infecting the gills were determined respectively. Additionally, copepod preference for specific hemibranchs and specific position and orientation on a hemibranch were also examined. Results indicated that *Nemesis lamna* individuals (males, females and combined) are unevenly distributed amongst the hemibranchs. Additionally they exhibited no significant horizontal or longitudinal distribution, but a relationship was found between their longitudinal distribution and orientation on the filament.

The role of parasites in abalone farming and notes on an enigmatic digestive gland protozoan

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Abalone is one of the world's leading shellfish products. The estimated production in South Africa for 2006 is 800 tons of meat worth R 180 million. Parasites pose threats to the production of abalone worldwide. *Labyrinthuloides haliotidis*, withering syndrome, *Haplosporidium* sp. and *Terebrasabella heterouncinata* are but a few problematic parasites and diseases that have been observed in the past. In 2001 a new parasite was discovered in the digestive gland of *Haliotis midae* farmed in the Western Cape Province. The aims of this project were to describe and classify this unique parasite based on its ultrastructure and DNA and to evaluate its potential threat to the abalone industry. Farmed abalone were collected from the Western Cape, as well as wild abalone from the Tsitsikamma National Park. Digestive glands of each sample were fixed for histology, transmission electron microscopy (TEM) and DNA analysis following standard methods. Fourteen of the 150 farmed animals were infected with this protozoan. None of the 34 wild abalone were infected. Histology revealed that this is a protozoan measuring on average 16.39 µm long by 7.65 µm wide, forming a relatively large parasitophorous vacuole within the digestive gland cells. In TEM micrographs organelles common to apicomplexans were observed, these included the endoplasmic reticulum, nucleolus and structures resembling an apical ring. Owing to low infection levels, no DNA sequences have yet been obtained. To date, no evidence has been found that this protozoan parasite poses a threat to abalone farming in South Africa.

WormBoss – A national Australian computer-based sheep worm control tool

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'WormBoss' (www.wormboss.com.au) is a website and CD-based information package designed to promote efficient and sustainable sheep worm control in Australia. It contains comprehensive, current sheep worm management information for farmers, teachers and consultants.

The Australian Sheep Cooperative Research Centre and Australian Wool Innovation Limited supported development of 'WormBoss', recognising that the national industry loses A\$300 million annually from internal parasites. Leading parasitologists from 6 Australian states (provinces) worked closely for 2 years to assemble and review extensive technical material. Writers, website designers and a communications company ensured that a high-quality professional product was launched in March 2005. Revision of its technical content and structure continues.

'WormBoss' comprises:

1. A decision aid tool ('Ask the Boss'). This guides producers towards making considered decisions about monitoring and treatment of sheep for worms in their specific region. Producers respond to questions to define farm location, class of sheep, time of year and status of worm egg counts and resistance to worm remedies. 'Ask the Boss' then either generates a report containing a management decision, refers farmers to more detailed sources of information, or advises that they seek professional advice.

2. An updated compilation of the extensive national knowledge on sheep worms and their management. This is found in 4 indexed, easily navigable sections, namely 'Worm Management', 'Know Your Drench', 'Know Your Worms' and 'Know Your Sheep'.

The principles of 'WormBoss', with technical details amended for local circumstances, might be usefully developed and applied in Africa, particularly for remote locations, where educational institutions and agricultural extension staff could readily access appropriate information for students and resource-limited farmers.

The ultrastructure of the Australasian gannet louse *Pectinopygus bassani* (O. Fabricius, 1780)

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The classification of external parasites such as lice is mainly based on chaetotaxy and descriptions of cuticular carinae and endocarinae, derived from light microscopy. Very little information has been gleaned from scanning electron microscopy (SEM). This study focuses on providing a detailed ultra-structural study to confirm previously described characteristics and to describe previously unrecognised characteristics of the Australasian gannet louse. Lice were collected from *Morus serrator*, the Australasian gannet, in the Tory Channel, South Island, New Zealand. Specimens were fixed in 70% ethanol, identified, routinely prepared for SEM and viewed in a Leica Stereoscan 420 at 10 to 15 kV. The typically philopterid head shows a rectangular ante-clypeus with a broad anterior hyaline margin, as well as 2 lateral longitudinal ridges terminating in 2 small notches on the straight transverse posterior margin. Antennae, protected by a blunt conus, and situated in a deep antennal socket, terminate in peg organs and are sexually dimorphic. A distinct eye, carrying an ocular seta, could be distinguished. Mouthparts are bilaterally symmetrical with well-developed labium and labial palps, mandibles and labrum. The mesothoracic spiracles are large with a slit-like opening. The 2nd and 3rd legs bear 2 pretarsal claws apposing 3 robust pretarsal setae. The 1st appendage is modified to carry a pair of pincer-like claws. Six pairs of abdominal spiracles are present on segments III–VIII, with the 2nd segment showing the stigmatal scar. The female gonopore is surrounded laterally and ventrally by combs of setae. None of the male specimens reveal everted parameres, or a pseudopenis.

The phylogeny and geographical distribution of the fish-parasitic isopod family Gnathiidae

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Phylogenetics describe the taxonomical classification of organisms based on their evolutionary history. As species evolve, the morphological features shared with their ancestors will either be conserved or lost. The aim of this study was to determine whether there is any correlation between phylogeny and geographical distribution of

members of the isopod family Gnathiidae. The 72 morphological characteristics designated by Cohen and Poore in their phylogenetic analysis of the family Gnathiidae in 1994 were assigned to 108 species representing the known 11 gnathiid genera. Three new elasmobranch gnathiids from Australia, 7 southern African species and the new genus *Tenerognathia* were incorporated in this study. The program PAUP 4.0 (Phylogenetic Analysis Using Parsimony) was used to generate cladograms and to establish morphological relationships between species. Primer version 5 was used to identify the specific characteristics responsible for species groupings. Results from PAUP 4.0 showed that the elasmobranch gnathiids form a separate grouping within the genus *Gnathia*, indicating that these gnathiids share unique characteristics. With the aid of Primer version 5, these characteristics were identified as the shape and length of the pylopod and the pleotelson. When comparing phylogeny to geographical distribution, it was found that within the genus *Gnathia*, species described from Australia, southern Africa, the Caribbean, Panamic-region and Indo-Pacific all formed separate clades, indicating a monophyletic origin in each geographic locality of this interesting group of temporary parasites.

Preliminary results on the biodiversity and systematics of apicomplexans infecting South African tortoises

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Knowledge of apicomplexans infecting the blood of African tortoises is extremely scanty. This is especially true for the tortoises *Kinixys lobatsiana*, *K. speki*, *K. belliana belliana*, *K. natalensis*, *Geochelone pardalis pardalis* and *G. p. babcocki* researched in this study. The study aims to: 1) isolate apicomplexan parasites infecting exotic and indigenous tortoises; 2) provide host and locality records of described and new apicomplexan species; 3) describe new parasites using morphometrics and DNA analysis; and 4) use acquired knowledge of apicomplexans as biological tags for the future identification of hosts. To date, 48 tortoises belonging to 2 species and 3 subspecies have been sampled. Blood smears, stained with Giemsa, are proving useful in morphometric analysis of apicomplexans, while blood fixed in Karnovsky's fixative for TEM will also be used to help distinguish parasite species. It is also intended that blood, blotted on filter paper, will be used for DNA analysis using proven techniques. A *Plasmodium* species has been found in 2/15 *K. lobatsiana* (13%), 1/3 (33%) *K. natalensis* and in 1/18 (5%) *G. p. babcocki*. A species of haemogregarine has been identified in 5/9 (55%) *K. b. belliana*, 2/3 (66%) *K. natalensis*, 1/15 (6%) *K. lobatsiana*, 2/3 (66%) *G. p. pardalis* and 1/18 (5%) *G. p. babcocki*. In none of these hosts was *Plasmodium* found concurrent with haemogregarine infections. It is hoped that these and future results may increase the knowledge of apicomplexans parasitising chelonian hosts in South Africa.

Molecular phylogenetic analysis of crocodilian blood protozoans of the genus *Hepatozoon*

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Historically the microscopic simplicity of parasitic protozoan organisms has meant that the fine details of their taxonomy and phylogeny can be confusing. One such example is the genus *Hepatozoon*, which has experienced many taxonomic re-evaluations since its description. Molecular biology and bioinformatic advances have made it easier to clarify the evolutionary relationship between organisms. Haemogregarines matching the morphological and morphometric description of *Hepatozoon pettiti* were observed in

blood smears taken from Nile crocodiles captured in the Okavango Delta. DNA was extracted from these stained smears, after which portions of the 18S rRNA gene were amplified using *Hepatozoon*-specific primers. Initial sequence data enabled the development of primers that permitted the amplification of the full length of the gene. Sequence analysis was performed using the Staden package and results were compared to published sequences from other representatives of *Hepatozoon*, using ClustalX. The phylogenetic position of *H. pettiti* was determined through the construction of trees using a combination of the neighbour-joining and bootstrap methods. The resulting trees demonstrated that *H. pettiti* possesses the closest evolutionary relatedness to *Hepatozoon* species infecting snakes, and then to other reptiles. Currently, smears of wild American alligators from Florida demonstrating a *Hepatozoon crocodinilorum* infection are being subjected to the same processes. Results from the research into the life cycle of *H. pettiti* in the Okavango showed that leeches infesting infected crocodiles contained the *H. pettiti* 18S rRNA target gene, thus demonstrating that leeches do acquire this blood protozoan when feeding on crocodiles and might thus be one of its vectors.

Tissue responses to juvenile *Gnathia pantherina* feeding on the gill septa of the puffadder shyshark, *Haploblepharus edwardsii*, in South Africa

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Gnathiid isopods are characterised by having free-living, non-feeding adults and 3 stages of parasitic juveniles that feed on fishes. The juveniles, because of piercing and serrated mouthparts, are able to attach effectively to fish surfaces, drawing blood and tissue fluids. Third stage juvenile gnathiids, identified as *Gnathia pantherina*, feeding on puffadder shysharks (*Haploblepharus edwardsii*) in southern Africa, have been examined using scanning electron microscopy and histological methods. Tissue responses to *G. pantherina* juveniles on the gill septa of *H. edwardsii* are reported. Loss of host cuticle and sloughing of epidermis were evident at septum attachment sites, exposing the dermis beneath; hyperplasia of adjacent epidermis and displacement of cartilage were also apparent. Host mucus, blood, inflammatory cells, dislodged fragments of host epidermis and dermis, lay around the cephalon and pereopods of feeding gnathiids. Upper layers of exposed septal dermis were compressed, lifted and separated from the lower layers by the gnathiid mouthparts, especially by the gnathopods and mandibles, which penetrated deeply into the dermis; bleeding into the deep regions of the dermis and the hypodermis occurred, together with infiltration of inflammatory cells. Fish blood occurred in the anterior hindgut of attached gnathiids and degraded fish blood was seen in their digestive glands, while it was likely that anticoagulant produced by gnathiid salivary glands prevented fish blood from clotting. It is concluded that if shysharks carry large numbers of *G. pantherina* juveniles, gill septum integrity may be impaired; since the septa support the gill filaments, respiratory gas exchange may also be affected.

Improvement of fungal formulations for tick control using oils and sunscreens

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The use of an entomopathogenic fungus as a possible bio-control agent for ticks has been studied in the past with promising results. The major obstacle to the use of entomopathogenic fungi in the field is the rapid inactivation of the spores by ultra-violet (UV) radiation. Attempts to protect entomopathogenic fungi from damage by UV radiation have not been very successful.

In this paper we present preliminary results of a new approach of protecting spores of *Metarhizium anisopliae* formulated in water and oil emulsion, using sunscreens (Everysun[®] and E45 Sun Block 50[®]). This strategy involved adding 1% and 3% sunscreen with sun

protection factor (SPF) values of 30 (EverySun®) and SPF50 (E45 Sun Block 50®) for spore survival.

Spores formulated in water and oil and exposed to UV radiation without addition of sunscreen were observed to have low germination in Sabourauds Dextrose Agar (SDA). When 0.1 ml spore suspension of 2×10^7 /ml was plated, only 4 and 10 colony-forming units (CFUs) survived in water and oil formulations, after 1 hr of exposure to UV radiation, respectively. Total viability was completely lost after 4 hours of exposure in water and 5 hours in oil, respectively. However, formulations to which sunscreens were added retained viability even after 5 hours of exposure to the UV radiation.

Sunscreen with SPF50 offered better protection against UV radiation damage than SPF30. Addition of 3 % sunscreen SPF50 offered the highest protection, with 5 and 8 CFUs surviving in water and oil, respectively, after 5 hours of radiation exposure. Addition of 3 % sunscreen to either formulation resulted in higher spore survival than the addition of 1 % sunscreen. In both formulations, spore survival was consistently higher in oil than in water formulations after exposure to UV radiation.

Investigating the role of T cell responses to IL-4 in *Leishmania major* and *Nippostrongylus brasiliensis* murine infection models

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T cell-specific IL-4 receptor-alpha deficient mice were generated to study the importance of IL-4 responsive T cells in *Leishmania major* and *Nippostrongylus brasiliensis* murine infection models. *Leishmania major* susceptibility is associated with polarised T_H2 responses driven by IL-4, while a polarised T_H1 response driven by IL-12 is protective. In contrast, a polarised T_H2 response is thought to be necessary for the elimination of *N. brasiliensis*. The cellular specificity of IL-4R α deletion was confirmed by quantitative PCR and FACS analysis. The abrogation of IL-4R α function was confirmed by proliferation and differentiation assays. In contrast to BALB/c and global IL-4R α knockout mice, T cell knockout mice were resistant during acute *L. major* infection. Resistance was demonstrated by lack of swelling in the infected area and the development of protective delayed-type hypersensitivity. This suggests that polarised T_H2 cellular responses may be responsible for susceptibility to *L. major*. Furthermore, the beneficial role of IL-4 signalling in non-T cells was revealed.

T cell specific IL-4R α knockout mice could successfully clear *N. brasiliensis* infection as BALB/c mice, but demonstrated less severe lung pathology, while global IL-4R α knockout mice showed delayed worm expulsion. Successful clearance was demonstrated by lack of worms in the intestine and decreased egg numbers in the faeces. These unexpected results demonstrate the successful clearance of *N. brasiliensis* despite an impaired T_H2 response. In conclusion, T cell responses to IL-4 are responsible for susceptibility to *L. major* infection and are not essential in the clearance of *N. brasiliensis*, but cause hypersensitivity to this nematode. By targeting IL-4R α signalling specific to T cells, a better understanding of the immune mechanisms involved in diseases will assist in developing effective cytokine therapy to achieve immunity.

Parasites and tourism

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As the scope of international travel expands, an increasing number of travellers are coming into contact with parasites not endemic in their countries of origin. Exposure of travellers to health risks depends on the destination, travel characteristics and duration of stay. The risk is greatest for those travelling to developing regions

in tropic and subtropic countries where health standards are decidedly substandard. Malaria is the most common parasitic infection encountered by travellers. Intestinal protozoa are the most common infecting organisms identified in travellers with chronic diarrhoea. Other pathogens, normally confined to the tropics, are increasingly diagnosed in travellers. Cases of New World mucosal and cutaneous leishmaniasis have recently been encountered in tourists in Britain returning from South America. A variety of helminths are found particularly among the fish-eating populations of Southeast Asia. *Gnathostoma spinigerum* and various trematodes have been diagnosed repeatedly in returning travellers. Increasingly adventurous eating habits of visitors are likely to contribute to an increase in incidence of reported cases.

Entomopathogenic fungi with potential for tick control: a comparison of laboratory and field observations

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The conventional method of tick control using chemical acaricides is fraught with several problems, e.g. environmental pollution, chemical residues in meat, milk products and in wool, development of tick resistance and the exorbitant costs. An alternative tick control method using entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* is being investigated. The fungi were cultured on Sabourauds Dextrose Agar at 25°C for 3 weeks. The spores were then harvested, suspended in water or oil emulsion and sprayed on ticks using hand sprays.

Spores of the entomopathogenic fungi applied on adult cattle ticks, *Rhipicephalus appendiculatus* and *Boophilus decoloratus* in the laboratory, induced significant mortality in both tick species. *Beauveria bassiana* induced a higher mortality in *B. decoloratus* than in *R. appendiculatus*, whereas *M. anisopliae* produced the opposite result. *Beauveria bassiana* and *M. anisopliae* spores sprayed on *R. appendiculatus* feeding on cattle in the field induced higher mortality than when applied on off-host ticks in the laboratory.

Engorged *R. appendiculatus* from cattle sprayed with *B. bassiana* and *M. anisopliae* and maintained either in the field (vegetation) or in the laboratory produced very similar mortality results. *Rhipicephalus appendiculatus* sprayed with spores of *B. bassiana* and *M. anisopliae* when feeding on cattle and thereafter maintained in the field exhibited significantly higher mortality than ticks infected off-host and maintained in the field.

Both *R. appendiculatus* and *B. decoloratus* are susceptible to the entomopathogenic fungi, *B. bassiana* and *M. anisopliae*. Mortality in *R. appendiculatus* was higher when ticks were infected with fungi on-host than when infected off-host and maintained in the field.

Oil formulations of both fungi induced significantly higher mortalities than their corresponding aqueous formulations.

Prevalence, intensity of infection and host specificity of fleas of some small mammals in selected areas around the city of Windhoek, Namibia

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A study was conducted at 4 selected sites in the city of Windhoek from April to July 2006 in order to determine the prevalence, intensity of infestation and host specificity of fleas on small mammals. Small mammals were live-trapped using Sherman traps and necropsied before collection of fleas. Fleas were processed using standard parasitological procedures and were mounted permanently onto slides using Canada balsam. Fleas were identified to species level.

A total of 61 small mammals belonging to 5 species, i.e. *Tatera leucogaster*, *Gerbillurus paeba*, *Thallomys nigricauda*, *Elephantulus intufi* and *Rhabdomys pumilio* were captured. One hundred and thirty six fleas belonging to 8 species, i.e. *Xenopsylla brasiliensis*, *Xenopsylla cheopis*, *Xenopsylla hirsuta*, *Xenopsylla trispinis*, *Dinopsylla ellobius*,

Dinopsylla zuluensis, *Epirimia aganipes* and *Listropsylla aricinae* were collected from infested hosts. The prevalence of fleas ranged from zero in *E. entufi* and *R. pumilio* to 50 % in *T. nigricauda*, 55.1 % in *T. leucogaster* to 61.1 % in *G. paeba*. The overall prevalence of fleas was higher in male (54.3 %) than in female (34.6 %) hosts. The intensity of flea infestation did not vary significantly between host species and between male and female hosts. *Tatera leucogaster* was infested by all flea species except *L. aricinae*. *Dinopsylla ellobius* and *X. trispinis* were only collected from *T. leucogaster*, while *L. aricinae* fleas were only collected from *G. paeba*.

Molecular mechanisms of drug resistance in malaria parasites

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Malaria remains the most dreadful parasitic disease worldwide. The disease is a threat across the world, even in non-endemic regions where it is spread through international migration patterns and tourism (airport malaria). Malaria is transmitted from person to person through the bite of a female *Anopheles* mosquito. Thus, the parasites have a complex life cycle that shuttles between a vertebrate host and an insect vector.

Africa stands out as the continent mostly affected by malaria, since it accounts for 400–500 million clinical cases every year. Ninety per cent of deaths due to malaria occur in sub-Saharan Africa, mostly among young children. Many children who survive an episode of severe malaria often suffer from learning impairments or brain damage. Pregnant women and their unborn infants are also particularly vulnerable to malaria, as the disease causes prenatal mortality, low birth weight and maternal anaemia.

Malaria is caused by protozoan parasites of the genus *Plasmodium*. The 4 types of human *Plasmodium* species which cause malaria in its various forms are: *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium falciparum*. *Plasmodium falciparum* is the most widespread and dangerous of the 4 species. If left untreated, *P. falciparum* can lead to fatal cerebral malaria and result in deaths, particularly in endemic regions. Treatment of this complicated parasitic disease is made difficult due to resistance of the parasites to most of the commonly applied drugs. Malarial resistance against some of the drugs comes about as a result of various genetic factors such mutations and recombinations. The considerable genetic variation brought about by mutations and polymorphisms in drug transport pathways allows for selection of drug resistance, hence impairing the effectiveness of the drugs. Drug pressure results in selection of resistant mutant parasites and the subsequent spread of the genes conferring resistance through the parasite populations. Chloroquine and the pyrimethamine/sulphadoxine drug combination known as Fansidar are 2 of the WHO-recommended drugs in the developing world. Genes that are involved in drug resistance to chloroquine and pyrimethamine/sulphadoxine (Fansidar) have been identified.

Resistance in chloroquine-treated patients has been associated *in vitro* with point mutations in 2 genes, *pfcr1* and *pfmdr*, which encode the *P. falciparum* digestive-vacuole transmembrane proteins PfCRT and Pgh 1, respectively. Mutations that are associated with resistance to pyrimethamine/sulphadoxine have been identified to occur in regions encoding the dihydrofolate reductase (DHFR) and dihydropteroate synthetase (DHPS), which are the target regions for pyrimethamine and sulphadoxine, respectively.

The potential of lectins in the prevention and control of ticks and tick-borne diseases

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The role of lectins in tick vectors was investigated by comparing the presence of lectins in the haemolymph, guts and salivary glands of 2 tick species: (1) *Rhipicephalus appendiculatus*, the vector of *Theileria parva*, and (2) *Rhipicephalus pulchellus*, which is refractory to

this protozoan parasite. There was variation in the agglutination titres with erythrocytes from bovine, mouse and rabbit erythrocytes, suggesting different binding affinities and quantities of lectins. The haemolymph from *R. pulchellus* gave the highest agglutination titre with mouse erythrocytes (1024), compared to that obtained with rabbit cells (8). No agglutination was observed with bovine erythrocytes at all when assayed with the gut, salivary gland or haemolymph lectins from either tick species. The gut lectin from *R. pulchellus* also demonstrated high titres (1024) with rabbit cells, but moderate with mouse erythrocytes (256). Haemolymph from *T. parva*-infected *R. appendiculatus* gave double (512) the quantities of lectins compared to uninfected ticks (256) with mouse erythrocytes, suggesting an increase in the amount of lectin in the presence of infection and implying a role of lectins in the immunity of these arthropods.

The lectin from the gut of *R. appendiculatus* agglutinated purified piroplasms, a stage in the *T. parva* life cycle ingested by the tick when it feeds on infected cattle. The anti-lectin antibodies conjugated with Fluorescein isothiocyanate (FITC) bound on to the piroplasms, suggesting a possible point at which intervention may be developed for the control of theileriosis. The significance of lectins in tick parasite transmission lies in the fact that these molecules play an important part in cell-to-cell adhesion. Defining their binding specificities and quantities in a given tick species may lead to development of a novel type of disease control whose mode of action would be based on competing for the ligands for binding to the pathogen's receptors or preventing adhesion to host tissues (transmission-blocking), thereby preventing infection.

This study revealed that tick-derived antigens in the form of lectins produced antibodies that recognised the piroplasm as well as schizont stages of *T. parva*. With increasing attention on vaccination of cattle against ticks, there is a need to identify and show target antigens within the tick. The study has shown 3 targets, the salivary gland, haemolymph and the gut. It forms one of the 1st reports on the effect of lectin antibody on the development and transmission of *T. parva*. This also means that lectins may be used as disease immunogens that can possibly intervene at the piroplasm and schizont stages.

Pentastomid parasites in the Cambrian, 500 million years ago – an exceptional view of a small group of arthropods that today are endoparasites of land tetrapods

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Fossil parasites are extremely rare. One exception is the Pentastomida, a group of 120 species of arthropods parasitising land vertebrates, occasionally humans. The 500-million-year-old fossils, the only known fossil tongue worms, were marine and not parasitic on land animals. They are exceptionally preserved 3-dimensionally, preserving even delicate morphological details and representing larvae of different stages. This permits in-depth comparison with living species and can also serve to discuss the long evolutionary pathway of a parasitic group, for the 1st time, based on observable historical evidence. The relationships of tongue worms remain controversial. One hypothesis, based on molecular and sperm data, sees the group close to certain parasitic crustaceans, another regards pentastomids as stem-lineage derivatives of Arthropoda *s. str.* (taxon encompassing all arthropods with compound eyes and segmentally sclerotised body and legs); this view rests on ontogenetic, morphological and anatomical data, e.g. of the nervous system and the fossil evidence. We hope that our new investigations of pentastomids can add substantially to the discussion about the phylogenetic relationships of these parasites and also to our understanding of the early evolutionary directions leading to today's parasite–host interactions. Our current working hypothesis is that pentastomids initially lived as ectoparasites, but in the gill chambers of marine early craniote chordates. Adaptation to life in lungs could, thus, have developed long before the tetrapod level of chordate evolution. Accordingly, the infection of different tetrapod hosts after their move onto land, was a smaller step than it appears at first sight.

The synthesis and characterisation of metal complexes with high biological activity against malaria parasites

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Malaria is one of the leading deadly killer diseases in the world with a death toll of more than 1 million a year, of which 70 % is in Africa. The attack on the disease is 2 pronged, the vector itself and the parasite transmitted. Yet the parasite continues to develop more and more resistance against conventional drugs. Hence, the great need for the search for more effective drugs. This paper describes the synthesis and characterisation of new metal complexes with strengths ranging from 1.4 to 172 times as strong compared to one of the current drugs used. The synthesis may simply be represented by the equation; $M + L \rightarrow ML$, where M = metal atom, L = a suitable ligand and ML is a biologically active complex. The brief description of the synthetic method, characterisation of the ML complexes by techniques such as elemental analysis, mass spectrometry, Fourier-transform infrared spectroscopy (FT-IR), and proton nuclear magnetic resonance (¹HNMR) will be discussed. The results of the biological tests will be presented.

Correlation between *Babesia rossi* genotypes and clinical babesiosis of dogs in South Africa

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Babesia rossi, an intra-erythrocytic protozoan, causes a severe, often life-threatening disease of domestic dogs. Dogs treated early for *B. rossi* infection usually recover from the infection, but dogs left untreated or treated at a later stage of infection seldom survive the infection. Dogs infected with *B. rossi* have varied clinical manifestations that can be categorised as uncomplicated (with a good prognosis) and complicated (with a poor prognosis). Different *B. rossi* isolates have previously been identified by typing of a polymorphic repetitive region from the gene *Babesia rossi* Erythrocyte Membrane Antigen (*BrEMA1*). This gene encodes for a phosphoprotein localised on the cytoplasmic surface of the infected erythrocyte. Two hundred blood samples were collected from dogs with babesiosis presented at the Onderstepoort Veterinary Academic Hospital (OVAH). DNA was extracted from 200 µl of venous blood collected in EDTA from each dog. The samples were screened for the presence of *B. rossi* DNA using PCR and the Reverse Line Blot (RLB). Positive samples were re-amplified using primers specific for the *BrEMA1* gene and different gene profiles were visualised by gel electrophoresis. Amplicons were then sequenced. Analysis of the *BrEMA1* amino acid sequence revealed that there could be antigenic diversity. Five different genotypes were identified and named *Babesia rossi* types 1–5. The genotypes were compared to the case data. Dogs that had a fair prognosis with appropriate intervention were mostly infected with *B. rossi* genotype 3, whereas dogs with poor prognosis even after intervention were infected with *B. rossi* genotypes 1, 2, 4 and 5. Our preliminary findings indicate that there might be a clinically important difference between the various *B. rossi* genotypes identified.

The life-cycle of a *Paradiplozoon* sp. (Monogenea: Diplozoidae) from the Vaal Dam, South Africa

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Paradiplozoon species are gill parasites of cyprinids with the adult form occurring as 2 monoecious plathyhelminth sanguinivores fused in permanent cross-copula. To date, *Diplozoon paradoxum* is the only

archetype belonging to this family with a complete morphological description of all life-stages. The present study was performed to determine the morphological development of a *Paradiplozoon* sp. inhabiting *Labeobarbus aeneus* and *Labeobarbus kimberlyensis*. Adult and larval diporpa stages of a *Paradiplozoon* sp. were collected between November 2005 and August 2006 and allowed *in vivo* to lay eggs. Deposited eggs were maintained until the ciliated oncomiracidia hatched. The morphology of the oncomiracidium, diporpa and juvenile stages was determined from *in vivo* observations and flat-fixed, whole-mount specimens using light, epifluorescence and laser scanning microscopy. Oncomiracidia hatched after 23 days through the egg operculum in a head-first peristalsis-like manner. The unique morphological characters included the muscular bicuspid valve at the base of the pharynx, a median excretory bladder and a pair of ballast vesicles within the prohaptor region. Immature gonads were observed at the base of the incomplete digestive tract of oncomiracidia and the subsequent maturation of the gonadal, muscular and integumentary structures was described for all life-stages. Attachment clamp development of the *Paradiplozoon* sp. is similar to that described for *Paradiplozoon rutili*. The adult stage appears after fusion of the reproductive and neuro-muscular systems of the 2 larval diporpa into a single individual. This study enables species identification of immature stages of the *Paradiplozoon* sp.

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 with a unique attachment organ, the opisthaptor, at the posterior end of the body. The objectives of this investigation were to identify and to study the morphology of various monogenean parasites on fish from the Okavango Delta, Botswana. Field collections of various freshwater fish species were made at different localities in the Delta. Fish species subsequently collected were anaesthetised using MS222, identified, measured and dissected. Gills were removed and examined for monogenean parasites with a dissection microscope. These parasites were collected and thereafter studied by light and scanning electron microscopy using standard techniques. Monogeneans from the following fish species were studied: (1) *Annulotrema pikei* (Price, Peebles and Bamford, 1969) and *Annulotrema* sp. from *Hydrocynus vittatus*, (2) *Annulotrema hepseti* Paperna and Thurston, 1969, from *Hepsetus odoe*, (3) *Schilbetrematoides* sp. from *Schilbe intermedius*, (4) *Cichlidogyrus botswanensis* Christison, 2002, from *Sargochromis carlottae*, (5) *C. zambezensis* Douëllou, 1993, from *Serranochromis robustus* and (6) *S. altus*; as well as (7) *Cichlidogyrus dossoui* Douëllou, 1993, *C. halli* (Price and Kirk, 1967), *C. sclerosus* Paperna and Thurston, 1969, and *C. quaestio* (Douëllou, 1993), all from *Tilapia rendalli*. Our results showed that a total of 10 different monogenean parasites were found to infest the gills of these 7 fish species in the Okavango Delta, Botswana. The monogeneans also demonstrated a high degree of host specificity as shown by other species around the world. Future studies in Botswana may reveal many more species, all of which may be used as bio-indicators to illustrate changes in freshwater environments.

Eye and cranial cavity diplostomatid metacercariae of freshwater fish in farm dams around Tshwane, Gauteng Province

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Most larval digenean parasites of the family Diplostomatidae use molluscs as their 1st intermediate host and fish as 2nd intermediate host. The objectives of this investigation were to study the morphology, prevalence and site specificity of 2 diplostomatid metacercariae. Freshwater fish species were collected using hand nets from the

Supersand Dam (close to Onderstepoort Veterinary Institute) as well as the Boekenhoutskloof farm dam (along the Moloto road). They were then dissected and all organs and muscle tissue were examined for metacercarial infections. These metacercariae were studied employing standard techniques using light and scanning electron microscopy. Diplostomatid metacercariae were found in the eyes of both *Pseudocrenilabrus philander* and *Tilapia sparrmanii*, whereas another diplostomatid metacercaria was found in the cranial cavity of *Clarias gariepinus*.

The prevalence of infection in all of these fish species was 100 % for both metacercarial types. Morphologically the results indicated clear differences in size and shape of the body, shape of the oral sucker and holdfast organ, and size and position of the acetabulum. The number of parasites in the eyes varied from 10–22 per host, with 8–10 cranial diplostomatids per host. These parasites may cause blindness or alter the behaviour of the hosts, and it is therefore imperative that the morphology, biology and effect on their hosts are studied. Previous studies showed that freshwater snails from these dams secreted 4 types of diplostomatid cercariae. In order to link these cercarial infections to metacercarial stages in the fish hosts, experimental life cycle studies should be undertaken.

A survey of raccoons, *Procyon lotor*, for raccoon roundworm, *Baylisascaris procyonis*, in western Pennsylvania, USA

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Baylisascariosis, the disease caused by *Baylisascaris procyonis*, the raccoon round worm, is an emerging infectious disease in the United States. When infecting humans, *B. procyonis*, causes visceral, ocular and neural larval migrans. Fatalities are common with neural larval migrans. During November and December, 2004, fur trappers provided the investigators with skinned carcasses of 52 raccoons, *Procyon lotor*, trapped in Indiana County, Pennsylvania, USA. Animals were sexed, small intestines were collected and examined visually for worms, a faecal sample was taken from the colon and examined *via* zinc flotation for eggs, and a forearm was removed from the carcass. Juvenile or adult age of each raccoon was determined using an X-ray of the wrist to establish the presence or absence of an epiphyseal cartilage band in the radius and ulna. The sample comprised of 29 male raccoons (20 adult, 9 juvenile) and 23 females (9 adult, 14 juvenile). Examination of raccoon small intestines revealed 30/52 (58 %) infected with *B. procyonis*. The male raccoon infection rate was 20/29 (65 %); while the female rate was 10/23 (43 %). The mean infection was 13 worms per infected raccoon. Zinc flotation of the colonic faecal sample showed 25 (83 %) of the 30 infected animals were patent (eggs passing in faeces). A χ^2 test was used to assess variation in *B. procyonis* infection levels between male and female raccoons and between adults and juveniles. No significant difference occurred between male and female infections. Juvenile raccoons had a significantly higher prevalence of 18/23 (78 %) of infection than adults 12/29 (41 %). Patency prevalence of 83 % is quite high and is probably related to the time of year that the specimens were collected; previous studies have reported incidence of infection to be highest in September–November.

The epifaunistic arthropod parasites of four-striped mice, *Rhabdomys pumilio*, in the Western Cape Province, South Africa

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Flea, lice, mite and tick species associated with 510 *Rhabdomys pumilio* were collected at 9 localities in the Western Cape Province, South Africa. The aims of the study were firstly to quantify the species richness, prevalence and relative mean intensity of infestation of epifaunistic arthropod species associated with *R. pumilio* and secondly to determine temporal variations in the mean abundance of the parasitic arthropods. Each mouse was examined under a stereoscopic microscope and its parasites were removed, identified and quantified. The epifaunal population comprised more than 25 000 individuals, and included 8 flea, 1 louse, 11 mite and 13 ixodid tick species. Female-biased sex ratios were noted for 30 % of the ectoparasite species. Three undescribed mite and 1 undescribed tick species were recovered and new locality records for 2 flea, the louse and 2 mite species were documented. A unique phoretic host association between a non-parasitic mite species, *Psylloglyphus uilenbergi kivuensis* and 3 flea species, *Chiastopsylla rossi*, *Hyposiphthalmus temporis* and *Listropsylla agrippinae* was recorded. The mean abundance of the parasitic mite and insect species was higher during the cold wet season, while ticks were more numerous during the warm dry months. The large number of ectoparasite species on *R. pumilio*, a locally abundant and regionally widespread species, is of medical and veterinary importance, particularly in relation to the transmission of pathogens such as *Anaplasma marginale*, *Babesia caballi* and *Babesia canis* to domestic animals, and *Rickettsia conorii* and *Yersinia pestis* and the viral disease Crimean-Congo haemorrhagic fever to humans.

Prevalence and genetic diversity of *Anaplasma marginale* strains in cattle in South Africa

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Bovine anaplasmosis, caused by the tick-borne rickettsia *Anaplasma marginale*, is endemic in South Africa and results in considerable economic losses to the cattle industry. This study was designed to characterise strains of *A. marginale* at the molecular level from cattle raised in communal and commercial farms in the north-eastern and southwestern regions of the Free State Province, South Africa, that varied in rainfall and vegetation. Seroprevalence to *A. marginale* was determined in 755 cattle by an *Anaplasma* sp. competitive ELISA (cELISA) and ranged from 44 % to 98 % and was similar in both regions. While *A. centrale* was not targeted in this study, *A. marginale* infections were identified by species-specific *msp1 α* PCR in 129 of 215 of the samples studied. Similar genetic diversity of *A. marginale* strains was found in both the northeastern and southwestern regions. The sequences of 29 *A. marginale* *msp1 α* amplicons from South African strains revealed considerable genetic diversity providing 14 new repeat sequences. However, 42 % of *msp1 α* repeat sequences were not unique to this region. These results indicated the presence of common genotypes between South African, American and European strains of *A. marginale*. Cattle movement between different parts of South Africa was suggested by the presence of identical *A. marginale* *msp1 α* genotypes in northeastern and southwestern regions of the Free State Province. Control

strategies for anaplasmosis in South Africa should therefore be designed to be protective against genetically heterogeneous strains of *A. marginale*.

Molecular characterisation of a *Babesia* sp. from sable antelope

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The sable antelope is regarded as a rare species listed as lower risk and conservation dependent on the IUCN Red List. Declining numbers could, however, lead to a 'threatened' listing in the near future. African antelope species are known to host a variety of intra-erythrocytic parasites; *Theileria*, *Babesia* and *Anaplasma* species have previously been reported from sable antelope. Recently, specimens from a sable that presented sudden onset of disease and subsequently died during immobilisation were submitted for molecular characterisation. DNA was extracted from blood samples, the V4 variable region of the 18S rRNA gene was amplified and analysed using the Reverse Line Blot (RLB) assay. RLB results showed that the PCR products did not hybridise with any of the *Babesia* or *Theileria* species-specific probes present, only with the *Babesia/Theileria* genus-specific probe indicating the presence of a novel species or variant of a species. The full-length 18S rRNA genes were amplified, cloned and the recombinants were analysed by sequencing analysis. Sequencing data were edited with the Staden package, aligned with published sequences of related genera using ClustalX and phylogenetic trees were constructed. Sequence similarity analysis indicated that a *Babesia* species infection was present, with highest similarity with *B. orientalis* and an unnamed *Babesia* species isolated from a bovine. Furthermore, a species-specific RLB probe was deduced from the hypervariable V4 region of the 18S rRNA gene, synthesised and will be used to screen samples to determine the prevalence of the parasites in sable antelope in South Africa.

Fractionation of parasite communities in relation to heavy metals and persistent organic pollutants

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Parasitism and level of contamination of gudgeon were studied at localities with different levels of chemical pollution (Bílina, Vlára and Ponávka Rivers, Czech Republic). The relationship among developmental instability (measured as deviation from perfect bilateral symmetry of 10 meristic and 8 morphometric fish traits), parasitism, concentration of selected pollutants in muscle and hepatopancreas of fish (mercury, nickel, PCBs, p,p'-DDE, etc.) and hepatopancreatic 7-ethoxyresorufin O-deethylase (EROD) activity, a biomarker of exposure of fish to dioxins and dioxin-like toxicants, were analysed. Detected levels of PCB, p,p'-DDE and some heavy metals (e.g. Ni, Zn, Hg) and EROD activity in the muscle and hepatopancreas of gudgeon differed among fish from different localities. However, there was no significant difference in the developmental instability of fish among studied sampling sites.

With the exception of 1 fish, all examined gudgeon were infected with metazoan parasites. Parasites of the taxa Monogenea, Trematoda, Cestoda and Acanthocephala were recorded and differences in parasite community composition among localities were found. Ectoparasites were the most abundant at all localities, whereas endoparasites were found with low mean abundance. The parasite community was fractionated into biologically meaningful components (generalists and specialists) and analysed in relation to concentration of pollutants and biological stress markers. A link between the EROD activity in gudgeon tissue and presence of either specialist or generalist parasites was found at all sampling sites. Fish

with a high exposure to dioxin-like toxicants were parasitised mainly by generalists; however, specialists were mainly found on fish with a lower exposure to dioxin-like compounds. Finally, there was no relationship between the concentration of pollutants and developmental instability of gudgeon at any of the examined sampling sites.

Comparison of TaqMan probe PCR using the Roche Lightcycler System with conventional PCR for the diagnosis of *Theileria parva* in South Africa

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In South Africa, Corridor disease is caused by *Theileria parva* transmitted by *Rhipicephalus appendiculatus* ticks from buffalo to cattle. Diagnosis of the carrier state of *T. parva* in buffalo is essential before translocation of buffalo. Conventional PCR assays have been used successfully for years, but the test is laborious, time consuming and requires the use of radioactive probes to increase sensitivity. A Real Time PCR assay based on TaqMan probe chemistry, was developed for the molecular diagnosis of *T. parva* and was compared to the conventional PCR assay. DNA was extracted from the blood of 8 gold standard positive animals, 50 known negative animals, 134 cattle and 443 buffalo from the field, using the MagNa Pure (Roche Diagnostics). The samples were tested for *T. parva* using the conventional PCR assay and on the LightCycler (Roche) using the TaqMan assay.

Results showed that the TaqMan assay was able to detect 5 more positive samples out of the 577 samples. DNA was extracted from serial dilutions of the blood from 2 experimental *T. parva* carriers – animals with either very low parasitaemia (0.02 %) or with no parasites detected on blood smears. The analytical sensitivity of the TaqMan assay was found to be 0.00001 % parasitaemia, which is equivalent to 1.2 parasites/2 µl. The TaqMan assay was found to cross-react with only *Theileria* species (buffalo) with a 9 nucleotide base difference from the *T. parva* 18S rRNA gene sequence. The biological identity (i.e. disease relations, host relations and vectors) of *Theileria* sp. (buffalo) has not yet been established. There are no cross-reactions with any other *Theileria* species, related haemoparasites, Gram-positive or Gram-negative bacteria. The TaqMan PCR assay appears to provide a rapid, highly sensitive and high throughput molecular test for *T. parva* diagnosis. Further investigations are required to increase the specificity of the TaqMan assay.

Myxosporean parasites of Okavango fishes

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Myxosporeans are parasites of lower vertebrates, of which fish are highly affected. These complex and microscopic endoparasites are causative agents of histozoic (e.g. in tissue) and coelozoic (in internal cavities) infections. Nonetheless, large aggregations of cysts in vulnerable organs such as gills and ovaries may compromise fish health. Cysts hold hard-walled spores, which are composed of 1 to 6 polar capsules containing extrudible polar filaments with an anchoring function. This unique morphology allows these parasites to survive unfavourable conditions and thus enables them to affect any organ of their hosts. For this reason the increasing worldwide fish production emphasises the importance of myxosporean research. The project objectives comprise investigating myxosporean species invading the internal tissue and organs of fish and identifying their taxonomic status. Fieldwork involved the collection of fish from the river system using handnets, gill nets as well as 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. Fifteen myxosporeans of 3 genera, *i.e.* *Myxobolus*, *Myxidium* and *Henneguya* were found to infect the liver, spleen, kidneys, intestine and gall-bladder of selected fish. Large cysts were mostly observed on the skin and buccal cavities of small fish species, whereas lower infections were detected in liquid media of compressed organs. Findings of this study produced 11 new myxosporean species which will add to the 13 previously described from this region. However, examination of other fish hosts will most probably result in even more undiscovered myxosporean species.

A survey of intestinal parasites in preschool children in Roma, Lesotho

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Intestinal parasitism in children is a public health problem worldwide and therefore should be periodically assessed. Epidemiological research has indicated that socio-economic and hygienic factors are important in the occurrence of these parasites in children. A survey was carried out in preschool children in the Roma valley to determine the prevalence and intensity of intestinal parasitic infections and risk factors important for the occurrence of these infections. Stool samples were collected from 82 children (aged 2–6) from 4 pre-primary schools. The modified formalin-ether concentration method was used to concentrate parasite eggs and cysts and the concentrates were observed as wet mounts using a light microscope. Demographic, socio-economic, disease-related and hygienic data of the children were obtained by use of a questionnaire. Bivariate analysis was done to determine associations between parasites and the different factors.

Only protozoan parasites were found in the study area. The overall prevalence was 29%. No significant differences ($r = 0.129$, $P < 0.05$) were obtained with regard to gender. The species found were: *Entamoeba coli* (15.8%), *Iodamoeba buschlii* (6.1%), *Giardia lamblia* (4.9%), *Chilomastix mesnilli* (2.4%), *Endolimax nana* (1.2%) and *Entamoeba histolytica/dispar* (1.2%). No associations were obtained between parasites and the risk factors investigated. It is possible that the occurrence of parasites was due to factors not investigated in the study such as the presence of animals in the home and conditions at school.

A survey of *Salmonella* spp. in bovine and ovine meat in the commercial meat abattoirs in Namibia

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The Central Veterinary Laboratory of Windhoek, Namibia, assessed the prevalence of *Salmonella* spp. in meat samples and swabs from bovine and ovine animals. Three commercial meat abattoirs in Namibia, namely Meatco Windhoek, Meatco Okahandja and Farmers' Meat Market were involved. Meatco Windhoek and Meatco Okahandja supplied bovine meat samples while the Farmers' Meat Market supplied ovine meat samples. The objective of this study was to determine the need for establishing a sustainable surveillance programme for *Salmonella* spp. in the commercial meat abattoirs in Namibia. The results of 6247 meat samples and 4875 swabs analysed at the Central Veterinary Laboratory for the presence of *Salmonella* spp. for the period of January and December 2002 were scrutinised. The occurrence of *Salmonella* spp. in swabs was found to be higher than that in meat samples. Similarly, the salmonella prevalence was higher in bovine meat samples than ovine meat. The percentage number of positive days in Windhoek abattoir was found to be higher on Wednesdays and Fridays while in Okahandja abattoir Tuesdays and Fridays had more positive cases than the other days of the week. Based on these findings, the microbiological quality of meat can be improved through education and training, inspection, microbiological testing and HACCP. The Directorate of Veterinary Services should collaborate with abattoirs and other bodies in conducting surveillance and research programmes

on the prevalence of different pathogens in the abattoirs. Introduction of a pre-harvest surveillance and intervention programme at the farm level would help in reducing the prevalence of food-borne pathogens at the post-harvest stage.

Genetic diversity of South African *Theileria parva* isolates

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Restriction fragment length polymorphism (RFLP) and sequence analysis of the variable regions of the parasite antigen genes *p67*, *p104* and *PIM* were analysed in search for discriminatory differences for differentiation of buffalo- and bovine-associated *T. parva* isolates. Cattle and buffalo blood samples from different areas in South Africa were investigated. The variable regions of the *p67* and *PIM* genes were amplified and analysed by RFLP. Amplicons from *p67* and *PIM* genes from selected isolates were sequenced.

The presence or absence of the 130 bp insert in the *p67* gene has been used as a marker to distinguish between cattle- and buffalo-associated *T. parva* isolates in East Africa. The larger *p67* PCR product was obtained from most buffalo isolates analysed. The KNP, Mabalingwe and Itala isolates yielded an additional smaller *p67* amplicon. The presence of both the large and small *p67* products from most buffalo-associated *T. parva* isolates indicates that this criterion cannot be used to distinguish between buffalo- and cattle-derived isolates in South Africa. Sequence analysis revealed 2 major groups of the *p67* gene. From 1 of the 2 groups, 2 subgroups consisting of buffalo- and cattle-associated *p67* genes were identified.

The *p104* profiles for the majority of the isolates from buffalo were typical of buffalo-derived isolates. *PIM* profiles from some isolates were homogeneous and resembled the Muguga isolate. *PIM* gene sequences from clones which appeared to share the same *PIM* profile were sometimes very different, implying that the *PIM* profile may not necessarily be an indication of the number of strains, or the heterogeneity, within an isolate.

It is evident that *p67*, *p104* and *PIM* PCR-RFLP profiles cannot be used to distinguish between cattle- and buffalo-associated isolates in South Africa. However, these profiles may assist in tracking *T. parva* infections in South Africa.

The associations between MHC genes and metazoan parasites in the fish populations living under different levels of environmental pollution

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The major histocompatibility complex (MHC) represents a group of genes of the highly polymorphic loci encoding the proteins that bind peptide fragments derived from the foreign antigens and stimulate an immune response. The specific role of MHC as a mediator of the adaptive immune response suggests that the MHC profile could reflect the effects of environmental changes connected to chemical pollution as well as the indirect parasite-mediated selection. However, until now MHC variation has not often been used in the pollutant assays.

In our study we tested whether the immunogenetic profiles measured by nucleotide and amino-acid substitutions in MHC class IIB (*DAB* genes) predominantly in peptide-binding regions vary between fish populations living under conditions of different environmental stress and parasite pressure.

In the freshwater fish gudgeon, *Gobio gobio*, 3 populations collected from 3 different localities, differing in the levels of environmental pollution (heavy metals and persistent organic pollutants) were studied. Fish were investigated for all metazoan parasites and representatives of Monogenea, Digenea, Cestoda and Acanthocephala were recorded. The differences in the presence or

absence of metazoan parasite species were observed as well as the parasite intensity infection that was different when comparing the 3 localities. Moreover, the different compositions of parasite communities were observed, specifically the proportions of specialist and generalist parasites. Even though several MHC haplotypes were shared between populations, specific alleles (or the specific nucleotide or amino-acid motives within alleles) were observed in each locality. The relationships between parasitism and MHC diversity in the association with different levels of environmental pollution were analysed and discussed.

The fish tongue-replacement isopod – Friend or foe?

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Ceratothoa imbricatus, a species of isopod belonging to the Cymothoidae, is found attached to the tongues of South African marine fishes such as the musselcracker (*Sparodon durbanensis*) and the blacktail (*Diplodus sargus capensis*). Whether this symbiont has a negative impact on its host is currently not clear, although it has been demonstrated that some species of *Ceratothoa* do alter the morphological development of the host's cephalon. The aim of this study was thus to determine whether this isopod affects the physiology and condition of its South African hosts. During April 2006, fishes were collected in the Tsitsikamma National Park and transported to a field laboratory where they were kept in aerated aquaria. In order to determine the effect of *C. imbricatus* on the metabolism of the hosts, the resting oxygen consumption of infested and non-infested fishes was measured in a closed respirometer. Results were subjected to a simple Student *t*-test. No significant difference was found between the oxygen consumption of parasitised and non-parasitised fishes. The effect of this parasite on the condition of its host was calculated by comparing total dry weight and total length of infested fishes with that of non-infested fishes. Again no difference could be found between parasitised and non-parasitised fishes. The only visible effect on the host was the complete dystrophy of the host's tongue. It is thus concluded that, although this isopod does damage the tongue of the host, it does not have a significant effect on the host's metabolism or condition.

The spatial distribution of *Kroyeria dispar* Wilson, 1935, on the gills of the tiger shark, *Galeocerdo cuvier* (Peron and Lesueur, 1822)

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Kroyeria dispar Wilson, 1935, is a parasitic siphonostomatoid occurring on the gill filaments of tiger sharks (*Galeocerdo cuvier*) together with *K. papillipes* Wilson, 1932. Although *Kroyeria dispar* shares several features with *K. papillipes*, it can be distinguished from other *Kroyeria* species by an unusually wide cephalothorax, absence of endopodal denticulations, presence of 2 pinnate setae on the medial margin of the 2nd endopodal segments of legs 1 and 2, denticulated endopod of the maxillule and cuticular flaps on the myxal area of the maxilliped. Furthermore, *Kroyeria dispar* can easily be distinguished from *K. papillipes* by the structure of the caudal rami. One complete set of gills of each of 14 tiger sharks caught in the nets of the Natal Sharks Board was investigated for infection with *K. dispar*. In order to investigate and analyse the spatial distribution of this copepod, the relationships between host size and gill surface area and gill surface area and number of copepods infecting the gills were determined. Additionally, preference by specimens of *K. dispar* for specific hemibranchs and specific position and orientation on a hemibranch were also examined. No relationship was found between the size of the host and the number of *K. dispar* specimens infecting it or the gill surface area and the number of *K. dispar* attached. *Kroyeria dispar* individuals exhibited some horizontal and longitudinal distribution on the different hemibranchs, but no significant orientation in relation with their distribution on the hemibranchs or filaments.

Benefits of urea-molasses block supplementation and tactical anthelmintic treatment of communally grazed indigenous goats in the Bulwer area, KwaZulu-Natal Province, South Africa

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KwaZulu-Natal (KZN) resource-poor farmers consider the poor reproductive performance of their communally grazed indigenous goats to be one of their major problems. Station-based studies at Pretoria indicated that dry-season urea-molasses block (UMB) supplementation of goats combined with the symptomatic treatment of anaemic goats for gastrointestinal nematode infection (Ivermectin 400 µg/kg) improved productivity, as based on carcass value. Anaemia was judged by examination of the colour of the conjunctival mucous membrane in comparison with a colour chart (FAMACHA[®] system). In a KZN-based field trial, 56 communally grazed indigenous goats were monitored every 4 weeks by FAMACHA[®], from February 2004 to October 2005. If judged anaemic they were symptomatically treated as above. Interestingly, in individual goats the degree of anaemia varied very little over the study period. This allowed the animals to be split into anaemic and non-anaemic groups, based on their FAMACHA[®] scores during the wet season in January 2005, when the worm burdens were expected to rise. Approximately half of the non-anaemic goats were tactically treated with Ivermectin at this time. Faecal egg counts indicated that this treatment successfully reduced the expected rise in wet season worm burdens. Approximately half of all the goats were also given UMB supplementation in the 2 dry seasons that occurred during the study period (May–September 2004 and June–September 2005). In symptomatically treated, anaemic goats the number of kids suckled per doe (KSPD) rose over the study period. UMB supplementation did not appear to have an additive beneficial effect on this parameter. In comparison, in non-anaemic goats, UMB supplementation combined with tactical treatment appeared to have a concomitantly beneficial effect on KSPD. Otherwise the KSPD remained relatively constant over the study period. The suggestion is that in communally grazed indigenous goats a regimen based on symptomatic treatment by FAMACHA[®] combined with dry-season UMB supplementation and tactical treatment of all goats in the wet season results in more kids being successfully suckled, hence improving the goats' reproductive performance. According to our figures, this could potentially mean an improvement of 35 % in the number of KSPD.

The internal and external parasite database 2005 for reserves in the Free State province

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Internal and external parasites were collected from various game species from 6 game reserves during the annual game capture starting in April 2005 until September 2005.

Faecal samples were taken and tested using the McMaster method. Gariiep Reserve had the highest internal parasite load, with the eland having the highest load at Willem Pretorius Reserve and the springbok the highest load at Gariiep Reserve, other reserves had very low loads of internal parasites. Most internal parasites were roundworms, especially *Haemonchus contortus*, except for the black wildebeest at Gariiep Reserve that had an infection of *Strongyloides papillosus*.

External parasites were collected in 10 % alcohol and examined

and confirmed. External parasites were collected directly from animals, as well as using cloth trains pulled through vegetation. *Rhipicephalus eversti eversti* was the most prevalent tick at all reserves along with *Hyalomma* species. *Haematopinus bufali* was collected from the buffalo at Koppies Reserve. Beside the general ticks, the highlights were the collection of *Hyalomma truncatum* collected from rhino at Willem Pretorius and eland from Koppiesdam, *Margaropus winthemi* from gemsbok at Willem Pretorius Reserve and from eland at Koppiesdam Reserve. All confirmations were done by I.G. Horak.

A morphological description of a *Diplozoon* species (Monogenea) on the gills of *Labeo rosae* in the Olifants River

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The family Diplozoidae consists of ectoparasites on the gill lamellae of cyprinid fish species. Very few parasites of the family Diplozoidae have been described from Africa and only 3 species occur in South Africa, of which 2 belong to the subfamily Diplozoinae, namely the *Diplozoon* sp. found on *Labeo umbratus* and a *Paradiplozoon* sp. found on *Labeobarbus aeneus* and *Labeobarbus kimberleyensis* in the Vaal River system. The other species belongs to the Neodiplozoinae subfamily, i.e. *Neodiplozoon polycotyleus*, found on *Labeobarbus* species in the Northern Province. This study is concerned with a 4th diplozoid species found on the gills of *Labeo rosae* in the Olifants River. The parasites were fixed flat with aceto-formaldehyde and preserved in 70 % ethanol. The morphological study involved the measurements of the specimens as well as whole mount staining through Hören's Trichrome and Fluorescent stains. The study of the whole mounts revealed the specimens' round opisthaptor bearing 4 pairs of clamps, an intestine with an unbranched, round ending, eggs with filaments as well as its reproductive system, which is confined to the fused region. Comparisons were made between this parasite and the other diplozoid species described in South Africa and it was determined that it is an undescribed species. Its distinct morphological characteristics such as its specific sclerite morphology, unbranched intestinal ending and eggs with filaments distinguish it from previously described species in South Africa.

POSTER ABSTRACTS

Ecological parameters of *Lamproglena hoi* Dippenaar et al., 2001 (Copepoda: Lernaieidae) infection on the Bushveld Smallscale Yellowfish, *Labeobarbus polylepis* (Boulenger, 1907)

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The genus *Lamproglena* was established by Alexander von Nordmann in 1832. All the species of this genus are gill parasites of freshwater fish, except *Lamproglena lichiae* Von Nordmann, 1832. Five of the 37 species have been recorded from southern Africa.

Lamproglena hoi Dippenaar et al., 2001, was the most recently described species and was collected from the Spekboom River in Mpumalanga on *Labeobarbus marequensis* (A. Smith, 1841) as well as *Labeobarbus polylepis* Boulenger, 1907. No ecological data are available on this parasite. This study aims to contribute to the understanding of infection by this parasite.

Bushveld smallscale yellowfish, *Labeobarbus polylepis*, were collected during June 2006 from the Pongola and Assegai Rivers, Mpumalanga, South Africa, and examined for the presence of parasites. *Lamproglena hoi* were collected from the gill filaments and fixed in a prostrate position with warm acetoformaldehyde alcohol and preserved in 70 % ethanol. The identification of parasites took place in the laboratories of the University of Johannesburg. Twenty-five copepods (prevalence 20.69 %, mean intensity = 4.17, abundance = 0.86) were collected from 29 fish in the Pongola River and 46 copepods (prevalence 40.00 %, mean intensity = 3.83, abundance =

1.53) were collected from 30 fish in the Assegai River.

New localities are reported and as well as the high prevalence, mean intensity and abundance of the parasite during winter.

A survey of plant-parasitic fungi occurring on Namibian *Acacia* species

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Over the past few years an increase in *Acacia* trees showing disease symptoms has been observed in the Windhoek Municipality area and the rest of the country. The importance of indigenous *Acacia* sp. to the Namibia flora is apparent considering that Namibia has 2 old deserts within its borders. These *Acacia* trees provide vegetation cover and browsing food matter to domestic livestock and wild animals. It is therefore crucially important to ensure that this vegetation is kept healthy. This survey is the first dedicated step to find ways of protecting them from disease-causing agents. The aim of this survey was to investigate the possible cause of disease symptoms on Namibian acacias. It is after understanding the biology of these fungi that a control method can be proposed.

The survey involved sampling plant materials (leaves, stems and roots) from dying trees showing symptoms like branch girdling, gum oozing and defoliation, suspicious general twig wilting and die-back. The survey was carried out in places where symptoms were observed. The *Acacia* species surveyed were *A. karoo*, *A. mellifera*, *A. erioloba* and *A. hebeclada*. Primary isolations from plant material and then single spore pure cultures were made for identification.

Preliminary results are reported. *Microsphearopsis* sp., *Dreschlerea* sp., *Botryosphaeria* spp., *Acremonium* spp., *Coniothyrium* sp., *Phellinus* spp., *Cytospora* sp., *Fusarium* sp., *Scytalidium* sp., *Phoma* spp. and *Gliomastix* sp. were isolated. *Trichoderma koningii*, *Poecilomyces variotii*, *Alternaria citri* and *Curvularia palescens* were also isolated from the diseased trees.

A comparison of the intestinal helminth communities of zebra species in southern Africa

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The intestinal helminth communities of 21 Hartmann's mountain zebras and 44 Burchell's zebras were compared using the original data from studies in South Africa and Namibia. Necropsy and worm recovery techniques were comparable between the studies. Thirty-six helminth species (20 genera) were recorded. There were significant differences in the helminth community structures of the 2 *Equus* species. Multiple confamilial species infections were the norm and no single-species infection was recorded for the Strongylidae. Congeneric species were commonly recorded in 3 genera (*Cyathostomum*, *Cylicocycclus* and *Cylicostephanus*). Geographic variation was apparent with a lower Strongylinae species richness in Burchell's zebras in Etosha compared to the Kruger National Park. Habitat sharing by Burchell's zebras and mountain zebras in the Etosha National Park contributed to host-switching and subsequent similarity in the helminth species composition.

A new genus and species of gnathiid isopod (Crustacea: Isopoda: Gnathiidae) from southern Africa

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Gnathiid isopods are unique crustaceans which fall in the family Gnathiidae. They are temporary ectoparasites, having 3 parasitic

larval stages that feed on blood and tissue fluids of teleost and elasmobranch fishes. Adults are free-living and are often collected in dredge samples. In South Africa, little is known about the biodiversity of these parasites, with the occurrence of only 5 species of gnathiids from the genus *Gnathia* Leach, 1814, and 1 species from the genus *Caecognathia* Dollfus, 1901. As part of a taxonomic study on gnathiids in the invertebrate collection of the South African Museum, Cape Town, a gnathiid species that does not conform to the other known species in South Africa or abroad, was found. Material used in this study was collected from several localities along the South African south coast between 1961 and 1972 and preserved in 70 % ethanol. Light and scanning electron microscopes were used to aid in identification and description of this species. The pylopod of the male, which defines Gnathiidae, consists of only 1 article which is unlike any other genus and has an additional appendage attached to it. The males of this species have a unique dentate mandible with 2 rows of unequal teeth on the blade and apex and there are 2 maxillules present which are usually absent on male gnathiids. These morphological characteristics distinguish this species from all the other 11 genera and this species is therefore proposed as a new genus and species.

Aspects of the feeding biology of *Lamproglena clariae* (Fryer, 1956)

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Lamproglena clariae is a well-known gill parasite of *Clarias gariepinus* attaching to the gills of the host using the maxillae and mandibles. Even though the mouthparts are not fully understood, it is generally accepted that feeding involves only the maxillae and maxillipeds. It is uncertain on which tissue type *Lamproglena* feeds, as blood is never apparent in the gut of *L. monodi*, whereas in the case of *L. clariae* and *L. barbicola* the gut is blood-filled. Therefore a study was conducted to examine the morphology of the digestive tract and the structure of the mouthparts using light and scanning electron microscopy.

Lamproglena clariae specimens were collected from *C. gariepinus* in the Vaal Dam after the fish were killed and the gills removed. The samples were fixed in an acetoformaldehyde alcohol solution and preserved in 70 % ethanol prior to dehydration and imbedding in resin. Serial sections were stained and a graphic reconstruction of the digestive tract followed. The digestive system consists of a fore, mid- and hindgut. A cuticle-lined, muscularised oesophagus extends into the midgut via a funnel-like structure. There are 3 zones within the midgut, namely the anterior, median and posterior zones characterised by the epithelial lining. The hindgut is a simple, cuticle lined tube. The digestive system morphology suggests that *L. clariae* is able to suck blood and this deduction is supported by the structure of the wound-inflicting appendages.

Helminth parasites of kudu, *Tragelaphus strepsiceros*, from mopani veld in Limpopo Province

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Wildlife and game farms contribute immeasurably to the economy, social well-being and conservation of biodiversity and ecotourism of the Limpopo Province. The loss of wildlife or poor growth rates due to parasitic diseases can be one of the major constraints to a profitable wildlife industry. Kudus (*Tragelaphus strepsiceros*), one of the more valuable antelope species on privately owned game farms in the Limpopo Province, was selected for a helminthological survey. Kudus have a wide distribution in southern and eastern

Africa and are regarded as a highly adaptable species, capable of exploiting a diverse range of habitats. They are mainly browsers that consume flowers, fruit, seeds, pods, leaves and twigs of a large variety of plants. The internal parasites of kudu were sampled seasonally from June 2005 to April 2006 on a game farm (2000 ha, with mopani veld dominating) in the Musina district. The entire gastro-intestinal tract, together with the heart, lungs and liver were removed within 2 hours after death. Standard collection and preservation methods were used for the different parasites from the various organs. The various aliquots of the ingesta and the entire digests and heart, lung and liver washings were examined in a Perspex counting chamber using a stereo microscope, and all parasites were collected. Nematodes were cleared in lactophenol, males identified to species level, females identified to generic level only. Cestodes were identified to generic level. The following helminths were recorded: *Elaeophora* sp. from the lungs (from a once-off survey at the Musina Nature Reserve); *Haemonchus veglii* from the abomasum; *Thelazia rhodesii* from the eye; and *Avitellina* sp. from the small intestine. Very low numbers of nematodes and cestodes were recorded compared to previous studies done on kudus in the Kruger National Park (where more than 100 000 nematodes were collected from 96 kudus). No trematodes were recorded during this study. The presence of parasites in game animals does not necessarily lead to noticeable disease(s) in the animals. Therefore, it is important to assess the type and level of parasitism in a population in order to recommend good management practices.

Adding to the few – A new, possibly raillietiellid pentastomid parasite from a gecko

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Pentastomida, a small, possibly stem-arthropod taxon with 120 living species, comprise exclusively (endo-)parasites of land tetrapods, including species living in animals of the African savannas. Pentastomids rarely infest humans, only species belonging to 3 taxa, by chance (yet, pentastomiasis is not always harmless, and may be lethal). We report here an apparently new pentastomid from the lung of a gecko from the Island of New Guinea. The characteristic head region with unequal appendages, the pair of large caudal outgrowths and the host taxon indicate that the specimen belongs to the taxon *Raillietiella*, but no described species of this or any other pentastomid genus possesses the lateral projections on the trunk of the newly discovered form. These projections appear, at first sight, appendage-like. Yet this is not the case because the trunk of extant pentastomids is actually not the somitic part but the non-somitic caudal end. This can be deduced from Cambrian fossilised and 3-dimensionally preserved stem-lineage pentastomids with tiny rudiments of limbs on the 2nd and 3rd true trunk somites (the 1st one is limbless) and the fact that ganglial knots of all 3 trunk segments are still left immediately behind the head portion and in the absence of legs. The annulation of the pentastomid trunk, therefore, is a pseudo-annulation. The function of the projections of the new pentastomid from New Guinea is still unclear. Our current effort is to discover more specimens for further investigations regarding morphology, anatomy and life history of this new form and its parasite-host relation.

Ecological aspects of *Bothriocephalus acheilognathi* Yamaguti, 1934 in the Vaal Dam

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Eighty largemouth yellowfish were collected during a seasonal study in the Vaal Dam. They were killed and the weight and length recorded. The fish were autopsied, the intestine removed and its length determined. Thereafter the intestine was opened in saline

solution and the attachment position of the 1st worm from the beginning of the intestine was measured and all the worms were then carefully removed from the intestine, counted and identified. These tapeworms were identified as *Bothriocephalus acheilognathi* Yamaguti, 1934. The prevalence, abundance and mean intensities were calculated. The seasonality and the relationship between the intensity of parasites in the intestine were compared to the intestine length and total length of each fish.

The position of the 1st tapeworm is 10 % to 20 % from the anterior end of the intestine, confirming that tapeworms lost their ability to metabolise fats and therefore located near the gallbladder. A prevalence of 100 % was recorded in all the surveys. The highest mean intensity of 231.1 was recorded in autumn, while the lowest mean intensity of 73.7 was recorded in the summer. The intensities recorded in the present study were higher than that recorded in 2000. The fish were in good condition and reproduction did not seem to be influenced by the tapeworms. Comparing intensities of the parasites to the intestine and fish lengths, no correlation could be found, indicating that older fish do accumulate parasites. Previous studies reported that the Asian tapeworm completes its life cycle in 1 year.

Efficacy and persistence evaluations of an injectable ivermectin abamectin formulation against an induced nematode and a natural *Rhipicephalus (Boophilus) sp.* tick infestation in cattle

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The efficacy and period of protection of a 2.25 % m/v ivermectin 1.25 % m/v abamectin combination injectable solution (Solution 3.5 % L.A.), administered via subcutaneous injection to cattle at a dose rate 1 ml per 50 kg body weight, were evaluated against a mixed induced nematode and a natural *Rhipicephalus (Boophilus) microplus* tick infestation. At the same time, the performance of Solution 3.5 % L.A. against *B. microplus* was compared with that of a registered 3.15 % ivermectin formulation. Results against nematodes were obtained by conducting a controlled test and by comparing untreated and treated group nematode burdens at necropsy. Results against *R. (B.) microplus* were obtained by performing tick counts at regular intervals. Solution 3.5 % L.A. was found to be effective against both immature and mature nematodes. The period of protection against a nematode re-infestation was found to be 56 days against *Ostertagia* spp., 42 days against *Cooperia* spp., 56 days against *Bunostomum* spp., 49 days against *Haemonchus* spp. and 56 days against *Oesophagostomum* spp. Efficacy obtained with Solution 3.5 % L.A. against *R. (B.) microplus* was not statistically different ($P > 0.5$) compared to that of the 3.15 % ivermectin formulation. Efficacy against *R. (B.) microplus* for both formulations lapsed between days 70 and 77.

Prevalence of *Entamoeba histolytica* and *Entamoeba dispar* among males and females in KwaZulu-Natal

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Reports of global incidence of 480–500 million were based on the misidentification of the morphologically indistinguishable *Entamoeba dispar* as *Entamoeba histolytica*. *Entamoeba dispar* is entirely non-pathogenic, while 5–15 % of *Entamoeba histolytica* infections are symptomatic. The true global incidence is 35–50 million, with an annual death rate of 40–110 000. Since the redescription of the potentially invasive *Entamoeba histolytica*, separating it from the morphologically identical non-invasive *Entamoeba dispar*, there is a need for the reassessment of epidemiological data on amoebiasis. In this

context we conducted a descriptive survey on the presence of *E. histolytica* and *E. dispar* in a rural area in South Africa. We found a high prevalence of the *E. histolytica/E. dispar* complex with microscopy, and electrophoresis revealed a considerably higher prevalence of *E. dispar/E. histolytica*, the most pathogenic amoeba for humans. One hundred Amoebic Liver Abscess (ALA) patients were recruited from King Edward VIII Hospital in Durban. Each of the 100 (ALA) patients recruited 10 close associates. The patients and the associates were enrolled into the study. Demographic and laboratory data were collected from the close associates of 100 of the index patients. Only specimens collected on the initial visits were analysed. Specimens were included in the data analysis (8.7 associates per patient). Informed consent was obtained and a short questionnaire completed to obtain demographic data and other relevant information. Stool samples were collected from the study volunteers following informed consent. Specimens were transported on ice to the Amoebosis laboratory, samples were labelled and Robinson/GM cultures were performed on all stool samples, electrophoresis was done on all cultures that had positive growths. The study found that *Entamoeba dispar* was more prevalent in the study area than *Entamoeba histolytica*.

A novel method of administering an endectocide, abamectin, in game

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Demand for endectocides for game has increased because of growth of the game-farming industry. A premix is not the best method to administer anthelmintics as game do not readily take medicated feeds. Efficacy of a novel method of administering an endectocide, abamectin, was tested in blesbuck (*Damaliscus dorcas phillipsi*).

Six blesbuck were treated, while 6 remained untreated. Capturing of the blesbuck was done by experienced, licensed game capture teams. Animals were allowed to adapt to boma conditions and artificial feeding before commencement of the trial. A pour-on was reformulated as a bolus to be fired onto the body of the animal by means of a compressed air rifle, similar to the ones used in paintball games. One bolus was applied per 100 kg body weight. The boli contained 0.5 % abamectin (w/v) and consisted of a gelatine capsule containing 10 ml compound.

Treated and untreated groups of animals were housed separately in an open-air boma. Six blesbuck were housed per boma. They received good-quality chopped lucerne and *Eragrostis* hay. Borehole water in troughs was available.

The animals were necropsied 7 days after treatment. Parasites in the abomasums of treated and control animals were recovered, counted and compared to calculate the efficacy of the formulation. No chemically-induced adverse reactions or injuries due to the application method were observed in any of the animals throughout the study period.

The formulation was 98.37 % effective against *Haemonchus* species present. The high efficacy and lack of adverse reactions clearly shows the positive potential of this form of treatment.

Remote treatment of ticks

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Efficacy of an acaricide containing 0.5 % abamectin (w/v) was tested when applied by means of a new application method. The product was reformulated as a bolus with gelatine capsule containing 10 ml of solution. The bolus could be shot onto the body of animals to be treated by means of a suitable compressed air rifle, similar to the ones used for paintball games. The rate of application was 1 bolus/100 kg body weight. Twelve weaners were allocated randomly to a treated and an untreated control group, each consisting

of 6 animals. *Boophilus* counts were done on each animal before treatment on day 0 and again on day 7. In each case the *Boophilus* species on the whole animal were counted.

The knock-down effect was determined by comparing the tick counts on the treated animals with the tick counts on untreated animals at the 7-day time point. The efficacy of the formulation against *Boophilus* was 98.32 %. No chemically induced adverse reactions or injuries due to the application method were observed in any of the animals throughout the study period. The high efficacy and the lack of adverse reactions clearly show that this form of treatment has positive potential.

Persistent acaricidal efficacy of ivermectin against 1-host ticks on cattle

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This study was carried out to determine the persistent efficacy of the test article against natural infestations of single-host ticks, after good knock-down efficacy has been established. Systemic treatments, with prolonged action against nematodes and ticks, will be of value to farmers.

Twelve cattle were allocated to 2 experimental groups, each consisting of 6 cattle. One group was an untreated control and the treatment group was treated with the test article (3.15 % ivermectin (w/v)) at 1 ml/50 kg body weight. The test article was administered intra-muscularly. Single-host ticks were counted *in situ* on the whole body on a weekly basis for 112 days. Percentage efficacies were calculated.

Results obtained showed that the formulation was still effective against *Boophilus* species at day 98, when the efficacy was 96.45 %.

No chemically induced adverse reactions were observed in any of the animals throughout the study period.

Cambrian Pentastomida – new finds contribute to understanding morphology and ontogeny early in the evolution of this group of parasites

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Exceptionally 3D-preserved larval pentastomids discovered between 1989 and 1994 from late Cambrian to early Ordovician rocks, *i.e.* c. 500 million years old, demonstrate striking morphological conservatism, but a high degree both of adaptation to parasitism and diversification already that require a likewise diversified host group. Much of the morphology and general shape of the larvae has remained virtually unchanged until today: *e.g.* the head region with paired sense organs, 2 pairs of hook-like legs for attachment to the host tissue, and a trunk comprising 3 segments and the caudal end bearing the anus and a pair of outgrowths. The fossils have a trunk bearing leg rudiments, which are absent in living forms. However, these latter forms have retained ganglial knots indicative of former legs and segments. New collections in 2004 yielded about 60 specimens of different sizes, currently being investigated.

First results demonstrate that ontogeny started with a free larva already having the limited number of adult segments. This suggests that pentastomids, not just the fossils, do not add segments, as other arthropods do, but their larvae just elongate considerably from moult to moult – at least 3 successive stages are known from our new fossil material. It also seems that pentastomids never became truly endoparasitic in their long life history, but retained, for example,, their layered cuticle and many other morphological characteristics, although their life cycle changed much more, even including adaptation to different successive hosts and an internal phase of the larvae in the host's body.