

Parasitological Society of Southern Africa

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Keynote Addresses

Understanding the role of gametocytes in the transmission of malaria and spread of antimalarial resistance

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Although eliminating the pathogenic asexual stages of *Plasmodium falciparum* is pivotal for the successful treatment of individual symptomatic patients, at a population level reducing the carriage of viable gametocytes is crucial for limiting the transmission of malaria parasites. Gametocytes are the non-pathogenic sexual stages of the *P. falciparum* parasite responsible for transmission of the infection from the human (or other vertebrate) host to the mosquito vector. The probability of a mosquito being infected depends on the duration and density of viable infectious gametocyte carriage in the human host, although immune responses also influence transmission. For *P. falciparum* there is a clear, albeit variable, relationship between gametocyte density and transmissibility. Antimalarial drug resistance spreads because of the increased transmission potential of resistant infections. The association between gametocyte prevalence and density (and thus predicted infectivity) with 1) pre-treatment asexual parasite density, 2) dihydrofolate reductase (dhfr) and dihydropteroate synthetase (dhps) mutation frequency, 3) clinical and parasitological response to treatment, and 4) artemisinin-based combination therapy was studied in South Africa and Mozambique. Increased gametocyte carriage was the earliest indicator of increasing sulfadoxine-pyrimethamine resistance, preceding a significant increase in asexual parasites or treatment failure rates. Relatively higher gametocyte carriage in the primary infection, as well as in the recrudescence infection, fuels the spread of the sulfadoxine-pyrimethamine resistant genotype. Artemisinin-based combination therapy was associated with highly significant reductions in gametocyte carriage. The implications of these findings on malaria transmission and the spread of antimalarial resistance will be presented.

Comparison of *Babesia rossi* and *Babesia canis* isolates with emphasis on effects of vaccination with soluble parasite antigens

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Babesia canis and *B. rossi* are large Babesia species that infect dogs and cause clinical disease. The spectrum of disease is highly diverse with either parasite, but upon evaluation of field cases it has been suggested that in general *B. rossi* is more virulent than *B. canis*. This difference was also found in experimental infections using *B. canis* and *B. rossi* isolates and appeared to be related to a difference in parasitaemia. Whether this difference reflects the essential difference between *B. canis* and *B. rossi* species in general, or merely reflects the variability in virulence of individual isolates can not be discerned. Comparative *in vitro* and *in vivo* studies revealed a number of qualitative differences between the *B. canis* and *B. rossi* isolates studied; however, more research is required to determine any causal relationship between *in vitro* and *in vivo* characteristics. Vaccination with a bivalent vaccine (containing soluble parasite antigen [SPA] from supernatants of *in vitro* cultures of *B. canis* and *B. rossi*) induced protection against clinical babesiosis upon challenge infection with either parasite. The dynamics of parasitaemia upon challenge infection of vaccinated animals indicated a biological difference between the *B. canis* and *B. rossi* isolates studied. Vaccinated dogs that were challenged with *B. rossi* parasites (2 isolates tested) effectively controlled parasitaemia. By contrast, vaccinated dogs that

were challenged with *B. canis* isolates (2 isolates tested) had little or no effect on parasitaemia but showed reduced levels of SPA in plasma. Apparently the nature of vaccine-induced immunity differs with respect to the challenge species.

Genes and health: The role of immune gene variability (MHC) in parasite resistance in fragmented animal populations

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Current discussions in conservation genetics focus on the relative importance of using selective neutral markers or markers of coding genes to identify processes of adaptive and evolutionary relevance in free-ranging animal populations faced by changing environmental conditions. In vertebrates, growing evidence suggests that genetic diversity is particularly important at the level of the major histocompatibility complex (MHC) because its gene products play an important role in immune functions. The central role of the MHC in terms of pathogen and parasite defense is undoubted (reviewed by Sommer 2005). We studied the importance of genetic diversity on resistance to gastrointestinal parasites in 10 populations of yellow necked mice (*Apodemus flavicollis*), a common rodent in Europe. The populations were sampled in habitat fragments of various sizes and differed in their levels of genetic diversity due to population size and gene flow. We found no evidence of an association between neutral genetic diversity measured by microsatellites and pathogen resistance amongst populations. However, the number of functionally important MHC-alleles within a population was significantly correlated with the parasite load. On an individual level, specific MHC-alleles were associated with high/low infection intensity. These alleles differed in mutations in the functionally important antigen binding sites. Similar results were observed in studies on the impact of fragmentation on the genetic constitution and parasite resistance in 2 lemur species (*Microcebus murinus*, *Cheirogaleus medius*) in Madagascar. The studies highlight the importance of adaptive genetic diversity in population health and conservation genetics.

Oral Presentations

Monogenea parasitising marine fishes in the Tsitsikamma National Park on the South Coast of South Africa

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The class Monogenea consists of small hermaphroditic ectoparasites with direct life cycles that are highly host specific. They are parasitic on marine and freshwater fish and are usually found on the skin, fins, nasal fossae and gills. They attach with a specialised posterior structure, the opisthaptor bearing hooks, hooklets, clamps or suckers. We investigated the monogeneans on the gills of selected marine fish in the Tsitsikamma National Park. All fish species were collected with hand nets and line in rock pools and lagoons. Fish were anaesthetised with clove oil whereafter the gills were removed. Monogeneans were fixed in 70 % EtOH or 10 % formalin and stained in ammonium picrate for light microscopy. Other material was fixed in gluteraldehyde for scanning electron microscopy. The following species were found to be infected with monogeneans: *Amblyrhynchotes honckenii*, *Diplodus capensis*, *D. hottentotus*, *Chirodactylus brachydactylus*, *Liza richardsonii*, *Rhabdosargus holubi* and *Sparodon durbanensis*. Morphological studies revealed that there were 9 different monogenean species, all of which were highly host

specific. The parasite load on each fish was low, ranging from 1 to 72 parasites per fish. None of the monogenean species found have previously been studied in this geographical location. The study indicated 9 unknown monogenean species from 7 fish hosts. Future studies on other fish species should be attempted in order to report on the diverse spectrum of monogeneans that seem to infest marine fish along the southwestern coast of South Africa.

Characterisation of South African *Theileria equi* and *Babesia caballi* isolates based on 18S rRNA gene sequences

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Genetic diversity amongst the protozoal parasites that cause equine piroplasmiasis was revealed during development of real-time PCR assays for the detection of *Babesia caballi* and *Theileria equi*. SimpleProbes were designed and used in real-time PCR assays to detect *B. caballi* and *T. equi* infections from 2 experimentally-infected ponies. *T. equi* infections could be detected from day 5, post-infection (p.i.) and *B. caballi* from day 8 p.i. The assays were further tested on samples that were positive in culture for either *B. caballi* or *T. equi*. The probes were, however, not able to detect target DNA in all of these samples. Parasite 18S rRNA genes were therefore amplified and sequence analysis revealed variation in the sequences, explaining the failure of the real-time PCR assay to detect all samples. The sequence diversity of the 18S rRNA gene was further assessed using samples representative of different geographical locations around South Africa. Whole blood samples were obtained and tested using the reverse line blot hybridisation assay. Samples that hybridised only to the *Theileria/Babesia*-genus specific probe and not to the *B. caballi* or *T. equi* species-specific probes were selected for further sequence analysis. Results indicated that extensive sequence variation occurs in the 18S gene of *T. equi* and *B. caballi* isolates. It is also evident that it will not be possible to design a real-time PCR assay to detect all *T. equi* and *B. caballi* isolates based on the 18S rRNA gene and that a different gene will need to be identified for that purpose.

Malaria in the concrete jungle

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Despite being a statutorily notifiable disease, malaria cases in travellers outside the 3 malaria provinces in South Africa are erratically reported and little is known about the burden of disease. Lack of awareness by both the public and health professionals may lead to late diagnosis and sub-optimal outcomes. Imported malaria is common in Gauteng. Over a 12-month period, 1705 patients with malaria were reported per a questionnaire directed at hospital admissions. The majority of patients had returned from Mozambique (85%). Where a diagnostic modality was reported ($n = 1665$), 98% of the malaria diagnoses were based on laboratory test results. Of 543 female patients, 39 (7%) were pregnant. 22.6% of patients were designated as severe. Thirty-seven patient deaths were reported (CFR 2.1%). In 776 cases (46%) a delay of more than 2 days was recorded, and 76% of patients did not suspect malaria. The majority of patients were treated with quinine as per the National Treatment Guidelines. Using laboratory data a further 4679 cases were identified during the first 6 months of the study period. A different category of malaria is that acquired by importation of infected mosquitoes. Gauteng receives a large volume of road traffic from risk areas inside and outside the borders of the country. Over an

8-year period we recorded 46 cases of malaria in residents who had not travelled to known risk areas, and we presume that most were manifestations of minibus taxi malaria. The case fatality rate was more than 10-fold higher than the national malaria case fatality rate. Medical practitioners should always be aware of the possibility of the diagnosis of malaria and travellers should be aware of the symptoms. Malaria should be looked for in any patient with otherwise unexplained fever and thrombocytopenia, even if there is no history of travel.

The use of multimedia in teaching veterinary helminthology

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Multimedia are currently used in the Department of Veterinary Tropical Diseases as an effective and interesting way of teaching Veterinary Helminthology to under- and postgraduate students. It is well suited to demonstrate helminth life cycles by using animation techniques and video. It is equally well suited to view the different morphological configurations of all 3 of the helminth orders by transferring micrographs taken with either a standard or stereoscopic microscope to multimedia software, such as Analysis™. The pathology caused by the various parasites can be added without too much trouble, provided 35 mm slides and/or video snaps of good quality are available. These are then scanned in and copied to Quest™ (slides) or converted to Flash™ format (video), and since Flash and Quest are compatible, a good quality product can be obtained. In this presentation the CD ROM that was produced for the training of post graduate students in the 'Helminths of Ruminants' module of the web-based M.Sc. is used as an example of how the different media can be integrated.

Prevalence of blood parasites in lions in Southern Africa

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Babesia is an intracellular erythrocytic parasite that occurs in various mammal species. The 2 most frequently reported species in felids are *Babesia felis*, which causes clinical babesiosis in domestic cats, and *Babesia leo*, primarily reported from asymptomatic lions. A recent study showed that *B. felis* and *B. leo* occurred more frequently in the host from which they had initially been described, but were also detected in other felid species. Identification of blood parasites using morphology alone can be misleading, while nucleic acid techniques such as the reverse line blot (RLB) can be of great value to differentiate between various parasites in the same animal. Blood specimens were collected from captive as well as free-ranging lions. DNA was extracted and PCR amplicons of the V4 variable region of the 18S rRNA were analysed by the RLB assay. Results showed that although a high number of samples tested positive for *B. leo*, a number of samples that were collected from captive lions also tested positive for *B. felis*. These 2 parasites also occurred as mixed infections in some samples. Specimens collected from lions in Etosha National Park, Namibia, tested positive only with the genus-specific probe, which indicated that these parasites, which morphologically resemble *B. leo*, had sequences in the 18S rRNA gene that differed from those of *B. leo*. These results are currently under investigation.

Mantoscaphidians (Ciliophora: Peritrichia) of gastropods in a marine environment

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Along the South African coast, several marine gastropods are host to a number of sessile peritrichs (Ciliophora) that belong to the genus *Mantoscaphidia*. These are *M. fanthami* found on various *Oxystele* species; *M. branchi* on all the limpet species and *M. midae*

and *M. spadicea* on *Haliotis* species, respectively. For the present study, giant periwinkles, also known as alikreukels, spiny chitons as well as 3 species of limpets were collected from intertidal pools during spring low tide in April 2007 from the De Hoop Nature Reserve. The gills of these gastropods were removed and smears were made to collect the mantoscyphidians. Wet smears were fixed in Bouins and stained with Mayer's haematoxylin to examine the nuclear apparatus. Gill material with ciliophorans was fixed in 2.5 % glutaraldehyde and prepared for the scanning electron microscope and fixed in 70 % ethanol for confocal microscopy. The objective of this study is 2-fold: (1) to determine the ideal preparation technique for these species and (2) to determine the role that confocal microscopy can play in (a) distinguishing between different species and (b) in describing morphological differences between populations.

The ultrastructure of a louse found parasitising the European bee-eater *Merops apiaster*

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The classification of lice is based on internal characteristics such as carinae and endocarinae, visible in cleared and mounted specimens as seen through the light microscope. This study intends to provide a detailed ultra-structural study to confirm previously described characteristics and describe characteristics previously not recognised. Specimens were collected from European bee-eaters on the farm Olifantskop in the Ellisras district, fixed in 70 % ethanol, routinely prepared for SEM and viewed in a Leica Stereoscan 420 at 10 to 15 kV. The head is exceptionally large compared to the body. The typical clypeus of the non-circumfasciate head was found to be very large but modified by a large anterior notch. Ventrally, the notch follows into a broad, shallow groove. Pulvilar lobes appear to be absent. The mouthparts are bilaterally symmetrical and mandibles appear more delicate and slender, with mandibular notches, shallow and longer than expected. The antennae appear typical in both sexes with plate, pore and peg organs clearly defined. The antennae are protected by an exceptionally large conus and large temporal regions. The prothorax appears much reduced and narrow, with the abdomen short and round, an indication that this is a body louse. Each leg bears 2 dorsal, opposed by 2 ventral, pretarsal claws. Six pairs of abdominal spiracles are present on segments III–VIII with only a stigmatal scar on the 2nd segment. The female gonopore is broad, flanked by a setae-bearing lateral flap on each side. None of the male specimens revealed everted parameters, or a pseudopenis.

Malaria Decision Support System Project

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Malaria is a vector-borne disease that causes extensive morbidity and mortality. Both morbidity and mortality have rightly received a lot of attention in monitoring of malaria control programmes. However, other indicators that are vital to the success of reducing morbidity and mortality have received less attention. This project looks at ways of integrating multiple indicators into a Malaria Decision Support System to facilitate informed decision and policy making for malaria control. Malaria vector control relies on the use of insecticide; Vector control programmes apply insecticides often on the basis of anecdotal data and/or general guidelines. The MDSS allows; i) Monitoring of entomological and epidemiological parameters related to disease transmission. ii) Improvement of vector control via prompt timely and focussed application of control methods. iii) Better informed decision making supporting policy changes for malaria control. Sentinel sites have been established in Malawi, Mozambique and Zambia to collect data for this project. As vector control with ITN or IRS relies on insecticides, at each locality insecticide resistance and resistance gene frequency are being monitored annually to aid insecticide choice. Species density and sporozoite rates are analysed monthly. This allows direct measure-

ment of the impact the control programme has on vector populations. An annual Malaria Indicator Survey helps establish the impact of the control programme on the human population. In order to support this work relevant spatial data are being sourced for geographical information systems. This allows visualisation and analysis of the information at an operational level by the malaria control programme.

Using GIS to understand malaria clusters

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The aim is to identify malaria clusters and heterogeneity of risk factors for disease transmission at town and village level. This will allow for informed decisions regarding focused interventions. Malaria clusters were determined over 3 seasons using SaTScan™, with specific reference to retrospective space–time permutation and the Bernoulli purely special model. Notified cases were investigated to obtain household information using field forms. Among the 7 towns investigated 4 significant spatial clusters and 2 temporal clusters were identified. One outbreak and 1 alert were confirmed by clusters. Bednet ownership was high (86 %) but usage low (63 %). Household walls constructed from mud were predominant (56 %). Thirteen per cent of cases were wrongly classified as local or imported. Relational data sets produced valuable information regarding spatial distribution of malaria risk and heterogeneity at sub-town level. Continuous monitoring of house structure is advised to ensure that appropriate insecticides for wall surfaces are procured and used. Any alternative or supplementary interventions will be dependent on a sound knowledge of local household characteristics and understanding focal malaria risk. Cluster analysis will allow for focused integrated malaria control strategies to be implemented.

Taxonomic review of the genus *Eupolystoma* (Monogenea: Polystomatidae)

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Polystomes are parasitic flatworms infecting mainly amphibians. Of these anuran polystome genera *Eupolystoma*, *Metapolystoma*, *Polystoma* and *Protopolystoma* have been collected in South Africa. The genus *Polystoma* has a worldwide distribution while *Metapolystoma* and *Protopolystoma* are limited to Africa and *Eupolystoma* is limited to Africa and India. The genus *Eupolystoma* currently contains 5 species. These species include *Eupolystoma anterorchis* parasitic in *Amietophrynus pantherinus*, *E. vanasi* from *Schismaderma carens* and *E. alluaudi* from *A. regularis*, *A. gutturalis*, *Nectophrynoides malcomi* and *Pyxicephalus adpersus* from Africa. The other 2 species, *E. chauhani* from an unidentified *Bufo* sp. and *E. rajai* described from an unidentified *Rana* sp., are both known from India. Specimens of *Eupolystoma* were recovered from the Mullerian ducts of *A. garmani*. Ducts infected with polystomes were swollen. In order to determine the pathological influence, infected ducts were histologically sectioned. Preliminary results indicate that *E. alluaudi* consists of a complex of different parasites. Owing to great intraspecific variation and limited interspecific variation, emphasis is placed on morphometric measurements of sclerotised parts, and in particular the marginal hooklets. A set of 13 measurements was taken of the sclerotised marginal hooklets of the *Eupolystoma* species by using Eclipse Net® software. The data were taxonomically analysed and 2 new species have originated thus far, resolving the taxonomic cluster of *E. alluaudi*.

Copper oxide wire particles in control of *Haemonchus contortus* in naturally infected sheep in Kenya

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The utility of copper oxide wire particle (COWP) boluses in reducing *Haemonchus contortus* infections was assessed in 2 groups of 24, 6 to 9 months-old, Red Maasai and Dorper crosses. COWP boluses were administered at 2 g and 4 g dosages to assess if they had an extended anthelmintic effect against incoming or established *H. contortus* infections. Within each group, 8 sheep were randomly allocated a 2g- or 4g-dose or were left as controls and were exposed to pasture infections before or after treatment with COWP. Two grams of COWP resulted in a faecal egg count reduction (FECR) between 47 and 31 % from weeks 4–6 post treatment in the group used to assess the effect of COWP on incoming larvae and between 92 and 44 % from weeks 2–6 post-treatment in animals that had established infections. The 4 g bolus had a FECR of between 84 and 44 % from week 4–6 post-treatment in sheep used to assess the effect of incoming larvae while a FECR of 96–50 % from week 2–8 in sheep already infected using the same dose. Significantly lower faecal egg counts were observed in animals treated with both doses of COWP in sheep which had established infections. No significant differences were observed in measurement of PCV in animals treated before or after infections. COWP had a limited effect on incoming larvae but had an extended period of effect on existing *H. contortus* infections. COWP boluses have the potential to be used as a dewormer by resource-poor farmers, as an adjunct in the control of *H. contortus* in sheep.

The micromorphology of the cattle tail louse *Haematopinus quadripertus*

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Lice collected from cattle in the Caprivi, Namibia, were identified as the cattle tail louse *Haematopinus quadripertus*. This obligative haematophagous louse is a tropical species reportedly causing cattle to become emaciated. As this species is difficult to distinguish from the shortnosed cattle louse *H. eurysternus*, it was decided to do a scanning electron microscopic (SEM) study in order to determine micromorphological characteristics of *H. quadripertus*. The lice were ultrasonically cleaned and routinely prepared for SEM and viewed in a Leica 420 stereoscan. The head was elongated with well-developed ocular points, and extended anteriorly to the distal haustellum which was surrounded by 4 pairs of long setae. The antennae bore several sensoria distally. The robust thorax was characterised by large notal pits, posterior processes, a pair of lateral mesothoracic spiracles and ventrally, a sternal plate with anterior processes. The 3 pairs of legs each ended in a robust tarsal claw which closed against a distotibial process. Two protrusible scaled pads further increased the potential to grasp the hairs of the host. The integument of the abdomen was membranous with rows of short setae and small sclerotised plates. Six pairs of bulbous paratergites which bore the abdominal spiracles and pairs of postspiracular setae, protected the abdomen laterally. A spiracular plate surrounded the spiracle lumen which was lined with pedunculate scales. The female gonopods VIII were lined with a fringe of setae, and lay adjacent to a small sclerotised genital plate. Gonopods IX had posterior-medial processes specialised to clasp the hairs of the host during egg-laying. The subgenital plate of the male had a species-specific shape with 4 anterior setae. These

observations may be of morpho-taxonomic importance in distinguishing these closely related species.

A SEM study of the adult *Varroa jacobsoni* mite from honey bees

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The adult mites were collected from bees at the USDA-ARS Carl Hayden Honey bee Research Centre, Tucson, Arizona, USA. These were identified as *Varroa jacobsoni*, which is not easily distinguished from *V. destructor* except by the size of the dorsal shield. As most descriptions of these haemolymph-sucking parasites have only made done using light microscopy, a scanning electron microscopic (SEM) study was done to investigate the micromorphology of these small mites which devastate honey bee colonies. Adult mites were routinely prepared for SEM and viewed in a Leica 420 stereoscan. The body of the female is ellipsoidal measuring 1500 μm wide and 1000 μm long. This dorsal shield was covered with feathery setae, except along the lateral margins where 23 movable hooked setae occurred. The ventral surface is protected by the sternal, genital, and anal shields medially and paired endopodal and mesopodal shields lateral to the legs. The shape and chaetotaxy of each of these plates were characteristic. The body of the male is smaller and more rounded. The legs of both sexes were short and ended in terminal membranous ambulacra which lacked claws. The tritosternum was bipartite and prominent. The mouthparts are made up of a pair of sensory pedipalps each bearing a 2-tined palpal claw. The chelicera lacked a fixed digit, while the movable digit in the female was lance-shaped for piercing with 2 denticulate processes for tearing. The chelicera of the male was modified as a tubular spematodactyl. The lumen of the stigmata were lined by sharp setae, while the peritremes wound along unique diverticular processes. These micromorphological structures may prove useful in future taxonomic investigations of *Varroa* mites.

Trichodinid ectoparasites of Anuran larvae

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Most trichodinids (Ciliophora: Peritrichia) are described from fish and are associated with fish hosts only. Some species are, however, also associated with other vertebrate hosts, such as amphibians (adult and tadpoles), as well as some invertebrates. One such species occurring on both fish and tadpoles is *Trichodina heterodontata* Duncan, 1977. *T. heterodontata* has an apparent affinity for cichlid hosts, but seems to be equally widely distributed on tadpole hosts. One of the objectives of the present study is to determine whether this species occurs on both tadpoles and cichlid hosts, or whether a different species is found on tadpoles in general. A further objective is to look at tadpoles and determine whether all tadpole species have the same trichodinid species. To determine the above, tadpoles will be collected from different locations in South Africa and Botswana. Skin and gill smears will be made and the trichodinids from the different tadpole species and populations will be compared with each other. The same will be done with trichodinids found on cichlids from the same locations as the tadpoles. A dental ring will also be used according to which 3 consecutive dentals are drawn as this method has proven itself to pick up morphological differences between populations. Fisher's least significant difference (LSD) will be used to determine if the variation between the different populations is significant enough for them to be described as different species. Tadpoles are seasonal and only present in water systems for a few months a year and this aspect will also form part of the overall study. Trichodinids can be found on tadpoles even in the absence of any fish hosts in a water body, raising the question where these ciliophorans come from and which hosts they occur on when the tadpoles have metamorphosed into adults.

The composition of the helminth fauna of helmeted guineafowls, *Numida meleagris*, from the Limpopo Province, South Africa

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Helmeted guineafowls are common in South Africa and widespread on the African continent. Despite this, few studies have been undertaken to elucidate the helminth fauna and its structure in South African hosts. During July 2005 and November 2006, 15 helmeted guineafowls were collected on a farm approximately 60 km west of Messina (Messina), Limpopo Province. The crop, proventriculus, gizzard, small intestine and caecum colon were opened in separate containers and their helminths collected. All the guineafowls were parasitised by helminths and harboured acanthocephalans, cestodes and nematodes concurrently. A total of 22 species was recovered from the alimentary canal, comprising 11 cestodes, 10 nematodes and a single acanthocephalan. Nine of the gastro-intestinal helminths were identified as core species, another 9 as secondary species and 4 species were classified as satellite species. While 4 nematodes, 1 cestode and the single acanthocephalan are considered generalists, many of the helminths have to date been recorded from guineafowl genera only and are recorded as specialists. This may change as more data become available. Seasonal changes in the helminth community structure seem linked with host ageing and changes in diet patterns, and sex does not influence the composition of the helminth community markedly. Host age is not an important determinant of community patterns, but some species were more numerous in young birds and some species had a higher prevalence in older birds. Guineafowls have a diverse helminth community and are usually tolerant of their helminth parasites.

Holy cows and sacred beetles – Sustainable parasite management on cattle farms

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Cattle farming forms a substantial part of South Africa's agricultural industry. Most cattle farmers use veterinary drugs on livestock in order to control endo- and ectoparasites. These antiparasitics enter the agro-ecosystem (either unchanged or metabolised) via the excretions of cattle. These residues may negatively affect non-target organisms, notably the beneficial dung beetles. In this presentation we review the biological importance of dung beetles and their ecosystem services: The dung burying activity of dung beetles leads to improved nutrient recycling, soil fertilisation, soil aeration, water infiltration, reduction of pasture fouling and reduction of dung-breeding pest and parasite populations. We will illustrate the dire consequences to an ecosystem suffering the loss of a functional dung beetle community by the example of Australia's cattle dung fiasco, which could only be solved by introducing dung beetles from other parts of the world (the majority from South Africa). We will explain our research focus on identifying parasite management options for cattle farmers in South Africa that are dung beetle friendly and sustainable. While these include various factors (including the diagnosis of pasture health and overgrazing), the central point of this talk will be our testing of veterinary drugs for their dung beetle compatibility. In short-term laboratory assays and long-term field trials we determine the lethal and sub-lethal effects of antiparasitic products on selected dung beetle species and the dung beetle community as a whole. Furthermore, we will explain the translation of our research results into an easy-to-use decision tool for farmers—our 'dung beetle friendliness trademark'.

Integrated control of ticks (Ixodidae) and tsetse flies (Glossinidae) in sub-Saharan Africa

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Vector-borne diseases, particularly trypanosomosis and tick-

borne diseases (TBDs) seriously limit livestock production and development and are major contributors to poverty in the continent. In addition, tsetse flies and ticks also transmit fatal human diseases such as sleeping sickness and tick-bite fever in many countries. The tsetse fly infests 10 million square kilometres of arable land in 37 African countries south of the Sahara. Babesiosis and anaplasmosis (transmitted by *Boophilus* species and biting flies), heartwater (transmitted by *Amblyomma* species) and dermatophilosis (associated with *Amblyomma* infestation) are present in all of the tsetse infested areas, while the tick *Rhipicephalus appendiculatus*, the vector of East Coast fever (*Theileria parva* infection in cattle), the major disease of economic importance, is present in 15 of the 37 tsetse-infested countries in eastern and southern Africa. Currently tick-borne diseases are controlled by the application of chemical acaricides to protect highly susceptible cattle through dipping, spraying or application of residual pour-ons. In much of Africa, regular tick control on indigenous cattle has not been practised against TBDs. The control of tsetse flies is also largely dependent on the use of chemical insecticides. Recently, there has been a shift towards using pyrethroid acaricides, which were originally developed to control ticks and at the same time are insecticides, to treat cattle as bait animals to control tsetse flies. Treated animals are introduced to graze fly-infested areas and tsetse flies are killed after feeding. The reports from different control projects have shown successful reduction in tsetse populations and, incidentally, reductions in tick burdens on cattle. However, the regular use of acaricide-bait cattle to control tsetse fly populations, with the opportunistic and unplanned killing of ticks has many disadvantages. This paper compares the ecological and epidemiological factors in the control of ticks/TBDs and tsetse/trypanosomosis. These factors include: the genetic resistance in cattle to vectors, endemic stability to TBDs and trypanosomosis, application of field immunisation, natural enemies for vector control, vaccines against vectors, diagnostics, and treatment. As no single existing or potential control option will provide the ideal means to control tsetse and ticks, an integrated approach is seen as the way forward.

An overview of the Oxpecker Translocation Project of the EWT Wildlife Conflict Prevention Group

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One of the EWT WCPG programmes concerns animal and environmental health. The objective of this programme is to achieve environmentally responsible animal husbandry. There are presently 2 facets to this:

1. Operation Oxpecker: achievement of this objective will be evident from an increase in the number of farmers using environmentally compatible ectoparasiticides and a natural expansion of the redbilled oxpeckers; into areas of their former range. The paper will present an overview of this project for 2006 and 2007.
2. The 2nd leg of this programme is Operation Dung beetle. This will look at endocides and is in collaboration with the University of Pretoria. A separate paper will be presented on this topic at PARS 2007.

A monogenean parasitising *Pseudocrenilabrus philander* collected from the Padda Dam

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Fish farming is a fast-growing industry in South Africa. *Pseudocrenilabrus philander philander* is aesthetically pleasing and could become an important ornamental fish. It is already utilised by subsistence fishermen in the Limpopo Province. The crowded circumstances in culture systems notoriously lead to an increase in monogenean infestations, with high mortalities and economic losses. A clearer understanding of the naturally occurring parasites of this fish species is therefore paramount. For the past 10 years a tiny monogenean has been recorded with a 100 % prevalence from southern mouthbrooders collected from the Padda Dam. Mono-

geneans are among the most host-specific parasites, following their respective fish hosts throughout their distribution range. Fish were collected with the aid of hand nets and transported to the laboratory, where they were killed by severing the spinal cord. Gills were inspected with the aid of a dissection microscope and parasite specimens were fixed and mounted in an ammonium-picrate-glycerine mixture. The ultrastructure was studied with the aid of a Zeiss compound microscope and photomicrographs taken using Axiovision software. Results were compared to that of type specimens of various species collected in Zimbabwe, 1 of which was from the same host as the present study. The unique structure of the copulatory complex of *Cichlidogyrus* is extremely species specific. The copulatory complex of the study species is identical to that of *Cichlidogyrus philander*. The morphological features of the dorsal and ventral bar, the ucinuli as well as the average body length approximately 300 µm confirms that in the present study *Cichlidogyrus philander* Doullou, 1993 parasitises *Pseudocrenilabrus philander philander* in the Padda Dam.

Detection of a *Theileria* sp. isolated from dogs in South Africa

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A *Theileria* species was detected by PCR in blood samples collected from dogs in the Pietermaritzburg area and was also found in dogs presented at the Outpatients Clinic of the Onderstepoort Veterinary Academic Hospital (OVAH), in the Pretoria area, South Africa. In the Pietermaritzburg area, 79 of the 192 samples were positive, while 3 of 1137 of the Onderstepoort samples were positive. Three positive samples from Pietermaritzburg were co-infected with *Ehrlichia canis*. PCR positive samples were further analysed by the Reverse Line Blot (RLB) and sequence analysis. Phylogenetic analysis of the 18S rRNA full-length gene sequences of 1 sample (VT12) from Pietermaritzburg and 2 samples from OVAH (BC281 and BC295) revealed a close relationship with sequences of *Theileria* species (sable). Clinical signs in the dogs that were examined at Pietermaritzburg and OVAH included an immune-mediated condition with severe thrombocytopenia. These findings identify a *Theileria* sp. in dogs for the 1st time in South Africa and add yet another microorganism to the growing list of haemoprotozoan parasites infecting dogs worldwide. The clinical significance of this infection in dogs is poorly resolved.

Patterns of ectoparasite aggregation in the 4-striped mouse, *Rhabdomys pumilio*

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We investigated the aggregation patterns of the full ectoparasite assemblage on a rodent species, *Rhabdomys pumilio*. Five hundred and ten mice were trapped at 8 localities during September to December 2003 and 2004. Sex, reproductive state and tail length were recorded followed by complete ectoparasite recovery and identification to species level. The parasite population comprised 8 flea species, 1 louse species, 11 mite species and 13 ixodid tick species. The Morisita index of dispersion was used to measure aggregation and was significantly larger than 1 for the flea-, mite- and tick higher taxa and for the individual parasite species, *Androlaelaps fahrenheitsi*, *Chiastopsylla rossi*, *Haemaphysalis elliptica*, *Laelaps giganteus* and *Polyplox arvicanthus*, that occurred at all 8 localities. There was no significant difference in the degree of aggregation between the individual parasite species. The aggregation was generally lower in sexually active male mice compared to sexually active females, although the finding was not significant. Mice with

longer tail lengths recorded significantly lower aggregation levels for the tick higher taxon ($P < 0.05$) compared to medium- and short-tailed mice. Temporal variation in aggregation was recorded for the louse species and the mite higher taxon with an inverse relationship between aggregation and mean abundance.

Monogenean species of *Quadriacanthus* Paperna, 1961 (Monogenea) on the gills of the sharptooth and bluntfoot catfish from the Okavango Delta, Botswana

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Quadriacanthus species are gill parasites of Siluriformes, (catfish) and are represented by 26 species throughout Africa. These species are equipped with a unique attachment organ, the opisthaptor at the posterior end of the body. The objectives of this investigation were to identify and study the morphology of monogenean parasites on *Clarias gariepinus* (sharptooth) and *Clarias ngamensis* (bluntfoot) from the Okavango Delta in Botswana. Fish were collected from different localities in the Delta using gill nets. Collected fish were anaesthetised with clove oil, identified, measured and the gills removed. The gills were examined for monogeneans using a dissection microscope. These parasites were collected and mounted in ammonium picrate whereafter the opisthaptor armatures and reproductive organs were studied by light microscopy. Additional material was fixed in gluteraldehyde for scanning electron microscopy. Two monogenean species of the genus *Quadriacanthus* Paperna, 1961 were found to infest *C. gariepinus*. These 2 species show distinct variations in the opisthaptor, especially the shape of the dorsal bar and the dorsal accessory sclerites. The most characteristic feature is the shape of the copulatory organ. *Clarias ngamensis* was found to be infested with 2 *Quadriacanthus* Paperna, 1961 species. These species differ from each other by the shape and size of the ventral and dorsal accessory sclerites, the dorsal bar and the copulatory organ. This study demonstrated 4 distinctive monogenean species from the sharptooth and bluntfoot catfish. All of these monogenean species also demonstrated a high degree of host specificity as shown by most other monogeneans in Africa.

Digenetic parasites from *Lymnaea natalensis* sheddings from the Tshwane area

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Lymnaea natalensis serves as the major intermediate host for the giant liver fluke, *Fasciola gigantica*. This snail was found to be the most abundant within the Tshwane area, thus playing an important part as 1st intermediate host in life-cycles of most other digenetic parasites. The aims of this research project were to study both the morphology and possible life-cycles of 7 different cercarial types that were shed by this snail. *Lymnaea natalensis* was collected from various water bodies around the Tshwane area whereafter the cercarial shedding was studied using standard light and scanning electron microscopy techniques. The following cercarial sheddings were studied, namely 2 types of xiphidio-, 2 types of strigeid-, an echinostomatid, a clinostomatid and an avian schistosome cercaria. Most of these cercariae are undescribed and their life cycles are unknown. Strigeid- and clinostomatid cercariae were found to utilise fish as 2nd intermediate hosts, whereas echinostomatid and xiphidio cercariae were mostly found to re-penetrate the same snail hosts. The definitive hosts for clinostome and strigeid forms are possibly piscivorous birds, such as cormorants and darters, whereas echinostome and xiphidio parasites on the other hand possibly mature in snail-eating birds like ducks. Avian schistosome cercariae are known to penetrate the definitive hosts (usually ducks or geese) directly. Subsequent life cycle studies would therefore be imperative in order to describe all the larval and adult forms involved within the life cycles of these lesser known parasites.

Virulence in mice of *Trypanosoma congolense* stocks obtained from buffaloes at Hluhluwe-Imfolozi game reserve in KwaZulu-Natal, South Africa

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Trypanosomiasis is a disease of humans and domestic animals caused by haemoprotozoan parasites of the genus *Trypanosoma*. In livestock, *Trypanosoma congolense* is the most pathogenic and causes a disease known as Nagana. Previous studies indicate that *T. congolense* exhibit variation in their virulence profiles, a feature which can be reproduced in mice. In Southern Africa, cattle kept in the vicinity of wildlife appear to develop more severe disease when infected with trypanosomes. It is known that wildlife act as reservoir of the virulent strains of trypanosomes suspected to be responsible for the severe disease in livestock. In order to test this hypothesis, 5 isolates of *T. congolense* were collected from buffaloes in KwaZulu Natal, RSA and tested for their virulence profiles in Balb/c mice. For each isolate, 10⁵ trypanosomes were inoculated intraperitoneally into 6 mice. One group of 6 mice was used as uninfected control. For each group, parasitaemia, packed cell volume (PCV) and time to death were monitored at regular intervals. The prepatent period, i.e. the duration of time to the first appearance of parasites in the blood as determined through Buffy coat technique, was 7.9 ± 1.4 days. In the 5 groups, the evolution of parasitaemia presented a single peak associated with a steep decrease of the PCV of the mice. The median survival time of mice infected with the 5 isolates ranged from 9 to 14.5 days. Additional studies will be conducted using trypanosome clones derived from each isolate, in order to determine more precisely the magnitude of variation in virulence of the trypanosomes infecting wildlife and livestock in KwaZulu-Natal.

The animal health industry and our environmental responsibility

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The size- and segmentation of animal health products sold in South Africa are analysed and the segments most likely to have a major environmental impact are identified. Within these segments, the factors determining the magnitude of the environmental effects are examined. The most significant groups of active ingredients used in Animal Health products are focused upon.

Flagship species affected by animal health products are listed with a closer look at oxpeckers and dung beetles and which products are most likely to affect them.

The role that Industry and the regulatory authorities could and should play to limit these effects are discussed.

The isolation and characterisation of a *Babesia bovis* stock from outbreaks on a farm in KwaZulu-Natal, South Africa

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Cattle farming forms an important part of South Africa's agricultural industry and is at risk of incurring severe losses due to babesiosis caused by either *Babesia bovis* or *B. bigemina*. This study focused on farms in the Swartberg region of KwaZulu-Natal, where, despite vaccinations, babesiosis outbreaks still occur. PCR tests based on 2 single-copy variable *B. bovis* genes (Bv80 and BvVA1) were used to determine if the outbreak was a result of vaccine failure

or field isolate breakthrough. Bv80 PCR products were sequenced to determine the relationship between size difference and sequence variation. In addition to this, the 18S rRNA V4 hypervariable region was sequenced for each strain to ascertain if changes in the Bv80 sequences were reflected in changes in the 18S rDNA sequences. Bv80 variable region profiles and 18S rDNA analysis indicated that the vaccine strain was not responsible for the outbreaks experienced. Current vaccination against babesiosis makes use of a live blood vaccine, an approach that has a number of limitations. The microaerophilous stationary phase culture technique allows for the *in vitro* cultivation of *B. bovis* parasites for prolonged periods, facilitating the isolation of secreted protein antigens with potential protective properties. *In vitro* cultivation of the vaccine and field strains according to published methods yielded limited success. Preliminary results using ALBUMAX[®] supplemented medium have shown promising results thus far. This adaptation of the Swartberg strain is the 1st step in the isolation of potentially protective antigens to be used for the development of a recombinant vaccine against bovine babesiosis.

Tick, fly, and mosquito control – lessons from the past, solutions for the future

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In order to continue to produce livestock in a sustainable fashion, it is suggested that what was used in the past will continue to form the mainstay of future control. For the foreseeable future, we must conserve what we have, and use it in combination with all the principles of integrated pest management: namely strategic and focused treatments of animals, environmental control of breeding sites, disease management (including the principles of enzootic stability), and resistant breeds. While new technologies, such as the development of vaccines both against the insect pest in some cases or the disease they transmit in others, and genetic engineering hold out some hope for the future; these are not sufficiently well advanced to permit wholesale application.

Pentastomid infection in fish intermediate hosts

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The vermiform pentastomids, also called tongue worms, comprise an ancient taxon of approximately 131 species. The adults of most species inhabit the nasal passageways and lungs of reptiles, such as snakes, lizards and crocodylians, while others are found in the air sacs of gulls and terns, the nasopharynx and sinuses of canines, or parasitise amphibian hosts. Pentastomiasis in humans is rare, but cases were reported from Africa, the Middle East and South-East Asia. The life cycle usually includes a vertebrate intermediate host in which larvae undergo several moults to reach the infective stage. They are named for the 5 structures near the anterior end of the body: the mouth (oral cadre), and 2 pairs of claw-like hooks. During parasitological surveys of fishes, infective larvae were recovered from the body cavity or swimbladder of different fish species. Larvae were fixed in 70 % ethanol and mounted in Hoyer's medium for measurement and identification. *Sebekia wedli* were recorded from Flag Boshielo Dam (Mpumalanga) from the body cavity of the mormyrid, *Marcusenius macrolepidotus* with a prevalence of 17.2 % and abundance of 0.28. The cysts had a yellowish colour and closely resemble the cysts of *Clinostomum* metacercaria. Larvae of 2 genera were recorded from dams in the Phalaborwa region (Limpopo Province) from the swimbladder of *Oreochromis mossambicus*, i.e.,

Alofia sp. and *Subtriquetra rileyi* with a prevalence of 15 % and abundance of 0.27. A single specimen was recorded from a dam in the Komatipoort region (Mpumalanga) from *Clarias gariepinus*. Larval infections have previously been reported from the Cichlidae, Cyprinidae, Cyprinodontidae and Poeciliidae. Infections recorded during this study from the Mormyridae and Clariidae represent new host records.

A review of *Myxobolus* species (Myxosporea) on African *Hydrocynus* species

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Hydrocynus species belong to the family Characidae and represent the well-known tigerfish in Africa. In spite of the increasing interest in myxosporeans infecting fish in Africa, the available data and knowledge on these parasites from *Hydrocynus* species are very limited. *Myxobolus hydrocyni* was the 1st species described from *Hydrocynus forskalii* by Kostoingue and Toguebaye (1994) in Chad. Later this species was also described from the gills of *H. forskalii* by Fomena and Bouix (2000) in Cameroon, and the gills of *H. vittatus* by Reed (2000) in the Okavango Delta, Botswana. This study focused on providing descriptions of *Myxobolus* species infecting *H. vittatus* in the Okavango Delta, Botswana. Fish were collected and transported to the camp where they were kept in aerated aquaria. Gills, skin and organs were examined for the presence of *Myxobolus* spores. Spores were studied live, photographed, and also stained with silver nitrate. Seven myxosporean species were observed from all organs, excluding the swimbladder, of *H. vittatus*. The *Myxobolus* species found on the skin and gills conform to the description of *M. hydrocyni* previously described from this system, but differ from the descriptions from Chad and Cameroon. The 6 remaining spores are not only new species, but also represent the first record for the host *H. vittatus*. In conclusion, *H. vittatus* was found to be infected with a diverse variety of myxosporean species.

An overview of the Endangered Wildlife Trust's Wildlife Conflict Prevention Group

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The Wildlife Conflict Prevention Group was officially established on 18 July 2007, as an evolution from a previous EWT Working Group, the Poison Working Group (PWG). The PWG was established in 1992 to address the large scale poisoning of birds of prey, cranes, storks, game birds and waterfowl, and to address the detrimental environmental impacts of certain pesticides and their applications. Deliberate poisoning of wildlife is one of the most pervasive and significant impacts on animal populations, and the impact is seriously underestimated in South Africa. An analysis completed in 2004 showed that pesticides kill up to 500 000 game birds and waterfowl in South Africa annually. It has proved difficult to quantify the damage caused by the incorrect choice of pesticide or incorrect choice of application method or both for alien vegetation clearing and control of invasive indigenous species. The result of these poor choices is that the populations of non-target species of plants and other organisms are reduced and if this is severe enough, ecosystem functioning is disrupted. The presentation will cover the work and projects in which the group is involved.

Poster Abstracts

Efficacy of 3 anthelmintics in communally grazed sheep as reflected by faecal egg counts in a semi-arid area of South Africa

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A survey was conducted on the occurrence of anthelmintic resis-

tance of nematodes in communally grazed sheep in semi-arid South Africa, from January to March 2006. In the herds belonging to 12 smallholder sheep farmers, the efficacies of albendazole, levamisole and closantel were tested by faecal egg count reduction tests where 80 % efficacy was considered a cut-off point for anthelmintic resistance. The results of the faecal egg count reduction tests showed more than 80 % efficacy with all the drugs used in most cases, but there were notable exceptions. In 1 case, closantel was 72 % effective, albendazole was 68 % effective and levamisole was 58 %, 60 % and 75 % effective, respectively. The occurrence of anthelmintic resistance in this farming sector is of concern and steps should be taken to prevent its further spread and development to avoid a situation developing as exists on numerous commercial sheep farms in South Africa where resistance is very common.

Theileria species infection in the Cape buffalo (*Syncerus caffer*) in 2 game parks in South Africa

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Corridor disease, caused by *Theileria parva*, is a controlled disease in South Africa. Cape buffalo are the natural reservoir hosts of this parasite, which is transmitted by *Rhipicephalus appendiculatus* and *R. zambeziensis*. Buffalo also appear to be the original hosts of 2 other *Theileria* species infecting cattle, the relatively benign *T. mutans* and the apathogenic *T. velifera*, both of which are transmitted by *Amblyomma hebraeum*. *Theileria buffeli*, which may infect cattle, and the hitherto uncharacterised *Theileria* sp. (buffalo) have thus far only been identified in some buffalo populations in the country. *Theileria* parasites usually occur as mixed infections and although the benign and non-pathogenic forms do not have any significant economic importance, they can interfere with the diagnosis of the pathogenic forms and therefore confuse their epidemiology. To screen for *Theileria* species that occur in South African buffalo, DNA was extracted from 198 buffalo blood samples originating from the Kruger National Park and the Hluhluwe-Imfolozi Park. The V4 variable region of the 18S rRNA gene was amplified and subjected to the Reverse Line Blot (RLB) hybridisation assay. RLB results demonstrated the presence of *T. parva*, *T. mutans*, *T. velifera*, *T. buffeli* and *Theileria* (sp.) buffalo, either as single infections or as mixed infections. In a number of samples 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. This warrants further investigation.

The evaluation of Avotan® Pour-on (0.5 % m/v abamectin formulation) against nematode and ixodid infestations in cattle

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The efficacy of a 0.5 % m/v abamectin formulation (Avotan® Pour-on) administered topically at a dose rate of 1 ml per 10 kg body weight, was evaluated against a mixed induced nematode infestation, an induced *Rhipicephalus (Boophilus) decoloratus* infestation and a natural mixed multi-host tick infestation. Results against nematodes were obtained by conducting a controlled test and by comparing untreated and treated group nematode burdens at necropsy. Efficacy was obtained against the dose limiting species *Cooperia* sp. Efficacy was also obtained against *Bunostomum* sp. *Oesophagostomum* sp. *Ostertagia* sp. and *Haemonchus* sp. Results against *R. (Boophilus) decoloratus* were obtained by conducting a stall test and comparing the daily number of ticks collected from the untreated and treated groups. The efficacy lapsed on Day 41, thus demonstrating a period of protection against *R. (Boophilus) decoloratus* of approximately 3 weeks. Knock-down efficacy results against *Rhipicephalus appendiculatus* and *Amblyomma* sp. were obtained by conducting counts on animals at intervals and comparing the counts with the pre-treatment counts. A knock-down efficacy of >80 % was achieved from Day 7 onwards.

Southern African marine fish siphonostomatoids

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Copepoda 'oar feet' animals are the most common and abundant metazoans in the sea. Worldwide there are more than 11 500 known species of copepods that belong to 9 or 10 different orders. More than 4224 are symbiotic and belong mostly to 2 orders, Poecilostomatoida (>1771 species) and Siphonostomatoida (>1840 species). The Siphonostomatoida consists of 37 families that are mainly marine with a third of the known species infecting invertebrates (20 families) while the remaining two-thirds infect vertebrates (17 families). To date, representatives of 15 of the 17 families infecting vertebrates (excluding Archidactylinidae and Tanypleuridae) have been recorded from southern African marine fish. The recorded families represent all of the clades in the estimated cladogram using morphological characters and the invertebrate associates as an outgroup. These 15 families include 64 genera and 188 species, a mere 10% of the known symbiotic Siphonostomatoida. The recorded specimens were collected from only 186 hosts (about 7% of known hosts). Considering the richness of marine fish species, estimated at 2500 in southern Africa with more than 2400 species just for South Africa, an extensive investigation of all possible fish hosts is bound to increase the number of recorded siphonostomatoids considerably and thus also our knowledge of a small part of the invertebrate marine biodiversity.

Dynamics of host–parasite associations between dung beetles and the dog nematode *Spirocerca lupi*

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There is a plethora of literature on the clinical aspects of spirocercosis in dogs, while very little deals with the host–parasite associations between dung beetles and *Spirocerca lupi*. Recently, there has been growing concern over the upsurge of reported cases of spirocercosis among dogs in South Africa, while little is known or understood about the dynamics of the host–parasite associations between dung beetles and this nematode. We conducted a pilot study during 2006 in the Tshwane (Pretoria) Municipality to determine and compare the prevalence of infection in dung beetles with the larvae of *S. lupi* between rural, urban and peri-urban areas. Prevalence of infection was significantly higher in dung beetles in the urban area (13.5%) compared with those in the rural area (2.3%). The current study aims to determine the prevalence of infection in dung beetles in 5 areas of high and low human population densities within 2 geographical regions (Tshwane (Pretoria) Municipality and Grahamstown) and compare the prevalence between the 2 regions. Sampling will be conducted 4 times over a breeding season using pig dung baited pitfall traps. Dung beetles collected from the traps will be identified to species level and dissected to confirm the presence or absence of *S. lupi* larvae. The parasite counts will be collated from the observations. We will also determine which species of dung beetles collected in each geographical region are susceptible to infection under natural conditions, since it is not known exactly which or how many species of dung beetles transmit this parasite. A better understanding of the dynamics of the intermediate host–parasite associations between dung beetles and *Spirocerca lupi* may contribute towards identifying management priorities for those with a technical, economical or legal and political interest in the problem, and the need for better control and preventative measures to be investigated for this disease in dogs.

The micromorphology of the warthog flea *Echidnophaga larina*

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Sticktight type fleas collected from a warthog near Bloemfontein

were identified as *Echidnophaga larina*. A scanning electron microscopic (SEM) study was made to understand the micromorphological characteristics of these fleas which enable them to attach, feed and reproduce on the almost hairless thick integument of the warthog. The fleas were fixed in 70% ethanol, routinely prepared for SEM and viewed in a Leica Stereoscan 420. *E. larina* had a characteristic angulate frons, a large metepimeron and a compact abdomen formed by compressed tergites and sternites. The head bore large rounded eyes just anterior to the antennal fossa containing the pedunculate antenna which bore 5 terminal setae. The maxillary laciniae were elongated with a rasplike toothed structure specialised for penetrating the thick epidermis of the host. The legs were well developed, with each terminal tarsus bearing a pair of elongated apical claws. The 5th tarsal segment bore 4 pairs of lateral plantar bristles, with the 2nd pair slightly longer and closer to the 1st than to the 3rd pair. This segment also bore a pair of strong pre-apical plantar bristles. The chaetotaxy of the 5th tarsal segment is used to distinguish the *Echidnophaga* species. The abdominal spiracles were not easily viewed due to the overlapping of the tergites. Those on the metepimeron had a spiracular plate surrounding an irregular opening through which spinous setae extended. The spiracles on tergite VIII were very elongated with a scalloped plate and many spinous setae forming the internal filter apparatus. The micromorphology of the posterior reproductive apparatus on tergite VIII including the sensillum, anal stylets, claspers and chaetotaxy were clearly recorded. These micromorphological structures may be useful in future comparative studies of related fleas.

Gyrodactyliasis – a cause for concern?

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Fish disease can be a significant cause of economic loss in the aquaculture industry. Disease is rarely a simple association between a pathogen and the host. Stress, the environment and the consequent management practices all play major roles. Previously we reported on a small parasitic flatworm (fluke) that was the cause of great losses in catfish production. Gyrodactyliasis refers to an outbreak of these monogenean flukes. The gyrodactylids are probably the most successful group of parasitic flatworms with regard to the number and kind of animals which are utilised as hosts. More than 400 species of the cosmopolitan genus *Gyrodactylus* have been described and 17 species are currently known from freshwater fish in Africa; with only 2 species, i.e. *G. transvaalensis* and *G. rysavyi*, described from *Clarias gariepinus*, from South Africa. Attachment with large anchors, together with subsequent feeding, damages the epidermis of the host, allowing secondary infection. The Gyrodactylidae are viviparous and give birth to fully developed adults. Intra-uterine embryos already contain 2nd and 3rd generation embryos. Four individuals are thus produced from a single zygote. This form of reproduction permits fast population growth and greatly reduces generation time. A severe case of gyrodactyliasis was identified after a commercial fish farmer from the Modjadje's Kloof region (Limpopo Province) received 4000 *Oreochromis mossambicus* fingerlings, 4 cm in length, from a fingerling producer. Fingerlings were raised in a 22 m³ recirculating system at 27 °C and fed with a balanced dry feed. Approximately a week after introduction, the fingerlings were concentrated at the surface with very little vertical movement, while some showed signs of rubbing and flashing. Within a day after noticing the change in behaviour, approximately 40% of the population died. Fish were examined microscopically and a heavy infestation (550–700+ gyrodactylids per fish) was identified on the skin of all specimens. Fish appeared pale with excess mucus secretion and epithelial proliferation. Severe secondary fungal infection, leading to finrot, was observed in most of the affected cases. A therapeutic salt treatment was recommended and no further deaths were reported but the cost of treatment, decreased growth during the period of recovery and loss in dead fish increased the production cost radically.

Skin and gill parasites of *Oreochromis mossambicus* and *Tilapia sparrmanii*

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Oreochromis mossambicus (Mozambique tilapia) and *Tilapia sparrmanii* (banded tilapia) are freshwater fishes of the family Cichlidae commonly found in most rivers and dams in southern Africa. They are an important food source for man and other vertebrates and are attractive aquarium subjects. The aim was to study the skin and gill parasites of naturally infected fish from 2 farm dams and the fisheries at Hartebeespoort Dam. Freshwater fish were sampled using hand and cast nets. In the laboratory they were placed in aerated tanks and fed on fish flakes. For examination, fish were anaesthetised using clove oil. Skin smears were made, gills removed and scraped onto microscope slides. Parasites were studied using standard light microscopy techniques and ciliates were stained with Mayer's haematoxylin and silver nitrate. The following ciliates were found on the skin of *O. mossambicus*: *Trichodina heterodontata*, *Trichodina acuta*, *Chilodonella hexasticha*, *Ichthyophthirius multifiliis*, an *Apiosoma* sp, and an *Epistylis* sp. A fungus of the genus *Saprolegnia* was also observed. Two ciliates were found on the gills of *O. mossambicus*, namely *Trichodina centrostigeata* and *I. multifiliis*, as well as a monogenean parasite of the genus *Dactylogyrus*. The following parasites were sampled from the skin of *T. sparrmanii*: *T. centrostigeata*, *Trichodina compacta*, *T. heterodontata*, *C. hexasticha*, an *Apiosoma* sp, a *Gyrodactylus* sp and a *Saprolegnia* sp. The gills were infected with *T. centrostigeata*, *C. hexasticha* and an *Apiosoma* sp. It is clear from the results that a wide diversity of parasitic ciliates, monogeneans and a fungus naturally occur on the skin and gills of these 2 freshwater fish species.

Metazoan parasites and health of *Clarias gariepinus* from Nwanedi-Luphephe Dams – preliminary results

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Fish parasites are sensitive to changes in the aquatic environment and the presence of certain parasites is a good indicator of the deteriorated health of fish and consequently a deteriorated environment. Chronic exposure to pollutants causes biochemical, physiological and behavioural host changes that ultimately can influence the intensity and prevalence of parasitism. Pollution can increase parasitism if the host defense mechanisms are negatively affected, thereby increasing host susceptibility. However, pollution can also decrease parasitism if the parasites are more susceptible to a particular pollutant than the host, or if pollution levels eliminate the suitable intermediate host. The parasite fauna of fish thus represent the result of the interrelationship between the parasites, their hosts and many interdependent influences of the environment. Parasites from *Clarias gariepinus* formed part of a research project on fish health and the diversity and correlation between pollution levels and parasites in the Limpopo River System. Metazoan parasites were collected from different sites from the Limpopo River and tributaries. We report on 1 winter survey from the Nwanedi-Luphephe Dams. Parasites were fixed using standard methods and stored in 70 % ethanol. A parasite index (PI) was determined. The results indicate that the water quality is good at the sampling sites of the Dams with low metal concentrations and TDS values. All the fish examined were in good health with no abnormalities (except for occasional discolouration) recorded. The following parasites were recorded: *Macrogyrodactylus congolensis* and *Dolops ranarum* from the skin; unidentified digenean cysts from the gills; *Glossidium* sp., *Polyonchobothrium* sp. and *Paracamallanus* sp. from the intestine; and *Contracaecum* larvae from the body cavity. The hypothesis that the PI for ectoparasites will be higher in unpolluted water was well supported for this survey, with a higher PI for ectoparasites than

endoparasites recorded. But, when dealing with moderate levels of pollution, parasite communities may be less informative as pollution indicators. The key to utilising parasites as bioindicators is a thorough knowledge of parasite biology, at the population as well as community level.

Diversity of myxosporeans from 2 fish species in the Okavango Delta, Botswana

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Some myxosporean parasites cause marked pathology in their fish hosts. Large aggregations of plasmodium cysts in vulnerable organs such as the gills and ovaries interfere with normal organ functions. This consequently compromises fish health, production and market value of fish in the aquaculture industry, therefore emphasising the importance of myxosporean research worldwide. The project objectives comprise investigating myxosporean species invading the internal organs of 2 fish species in the Okavango River and Delta in Botswana, and to determine their taxonomic status. Fieldwork involved the collection of fish from the Okavango River using gill nets, as well as rod and line. Fish were anaesthetised with clove oil, whereafter the internal organs were removed. These were compressed between 2 glass slides and the run-off liquid was examined for live spores using light microscopy. Results revealed the presence of 13 myxosporeans of 2 genera, *Myxobolus* Bütschli, 1882 and *Henneguya* Thélohan, 1892, from *Hydrocynus vittatus* Castelnau, 1861 and *Hepsetus odoe* Bloch, 1794. Six different *Myxobolus* species and 1 *Henneguya* species were found in the organs of *H. vittatus*, whereas 5 *Myxobolus* species and 1 unknown myxosporean were found to infect the internal organs of *H. odoe*. Previous studies in the Okavango Delta indicated the presence of myxosporean parasites only on the gills and skin of these 2 fish species. This study, however, is the 1st to show histozoic myxosporeans in the internal organs of tigerfish and the African pike from the Okavango system.

Characterisation of South African *Theileria parva* isolates by PCR-RFLP analysis of the parasite antigen gene, polymorphic immunodominant molecule (PIM)

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East Coast fever (ECF), caused by cattle-associated *Theileria parva*, was eradicated in the 1950s in South Africa (SA). However, Corridor disease, caused by buffalo-associated *T. parva*, still occurs and is a controlled disease. Successful discrimination of *T. parva* subtypes in South Africa is important for obtaining accurate data on the epidemiology of theileriosis in order to improve control strategies to protect livestock against the disease. In this study, restriction fragment length polymorphism (RFLP) analysis of the variable regions of the parasite polymorphic immunodominant molecule (PIM) was used to discriminate between *T. parva* isolates. DNA was extracted from cattle and buffalo blood samples collected from different geographical areas in South Africa, the variable region of the PIM gene was amplified and subjected to RFLP analysis. Selected amplicons were sequenced and the data analysed using phylogenetic methods. PIM RFLP profiles from some isolates were homogeneous and the majority of the profiles were typical of buffalo-associated isolates. A new profile was identified from the Welgevonden isolate, whilst that of cattle isolates from Ladysmith was similar to that of Muguga, a Kenyan isolate that causes ECF. Phylogenetic analysis revealed the presence of 3 groups and included sequences similar to known buffalo- and cattle-associated *T. parva* parasites and recombinant sequences. Recombinant sequences clustered together in a single clade. The Welgevonden isolate grouped with buffalo-associated PIM sequences and the Ladysmith isolate with the

Muguga PIM sequence. It is not known whether the Muguga-like PIM profiles or sequences are associated with pathogenicity, although there have been no incidences of ECF in South Africa since its eradication and classic ECF was not diagnosed in cattle on the Ladysmith farm

The geographic distribution and a taxonomic description of ticks in the Free State Province

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Surveys aimed at determining the geographic distribution of ticks in Free State Province have been and are being conducted as a follow-up to the earlier surveys completed in the 1940s. Moreover, tick species and the diseases they transmit, which apparently did not previously occur in the Free State Province, have recently been reported here. In the 1st phase of the project surveys are in progress on communal and commercial farms, buffalo ranches and in private and public nature reserves. The geographic distributions of the localities at which ticks are collected during the project will be compared with those obtained in the earlier surveys. This may reveal shifts in the distributions of ticks of medical and veterinary importance and also add to the data on the distributions of recently described species. A reassessment of the geographic distribution of ticks in the Free State Province has thus become a necessity. In the 2nd phase the taxonomy of all stages of development of 4 closely related species in the genus *Rhipicephalus* is being revised. This will facilitate the identification especially of their immature stages. The 4 ticks will be reared in the laboratory and their larvae, nymphs and adults will be redescribed in detail using both light and electron microscopy, thus creating a data set against which previous and current collections can be revisited and precise identifications can be ascribed. The combined output of the 2 projects should add to a more accurate inventory of tick biodiversity in South Africa.

Anthelmintic efficacy of copper oxide wire particles against artificial *Haemonchus contortus* infections in indigenous Zulu goats

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The occurrence of widespread resistance of *Haemonchus contortus* to all major anthelmintic groups has prompted investigations into alternative control methods in South Africa. One alternative is the use of copper oxide wire particle (COWP) boluses. To assess their efficacy against *H. contortus* in goats, 18 male indigenous faecal-egg-count-negative Zulu goats were each given c. 1200 infective larvae of *H. contortus* 3 times per week during weeks 1-2 of the experiment. These goats constituted an established infection group. At the beginning of week 7, 6 goats were each treated with a 2 g-COWP bolus given orally; 6 goats received a 4 g-COWP bolus each and 6 animals were not treated. A developing infection group was made up from a further 20 goats. At the start of week 1, a 2 g-COWP bolus was administered to each of 7 of these goats; a 4 g-COWP bolus to each of another 7 goats and no bolus was given to a further 6 animals. Each of the goats was given c. 400 *H. contortus* larvae 3 times per week during weeks 1-6. In week 11, all 38 goats were euthanased for worm recovery from the abomasa and small intestines. In the developing infection goats, both the 2-g- and 4g-COWP treatments were ineffective in reducing the worm burdens relative to the controls (which had mean burdens of 1051 worms). However, the 2 g- and 4 g-COWP boluses were 95 % and 93 % effective, respectively, in reducing the worm burdens of the established *H. contortus* infections when compared with the controls (which had mean burdens of 442 worms, $P < 0.05$). The COWP boluses have the potential to be used as an alternative to conventional anthelmintics for the control of established *H. contortus* infections in goats.