

PARSA 2011

The following are abstracts of papers and posters presented at the 40th Annual Congress of the Parasitological Society of Southern Africa (PARSA), 10–12 July 2011, Stellenbosch University, Stellenbosch, South Africa.

Invited Lecture:

IL-4R α dependent cellular responses and mechanisms underlying adaptive immunity to *N. brasiliensis*

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Disease resolution against primary helminth infections depends on TH2 polarisation via IL-4R α expression on non-haematopoietic cells, however, specific roles for IL-4R α expression in the rapid protective immunity to re-infection are not understood. Using mouse models unable to express IL-4R α or with T- or B- cell specific disruption of IL-4R α expression we examined the efficacy of host immune responses to re-infection against *Nippostrongylus brasiliensis*. Our results demonstrated protective immunity to *N. brasiliensis* to be mediated via IL-13 signaling through IL-4R α . Optimal killing of parasites following re-infection requires IL-4R α expression on T- and B-cells. Both T-cell and B-cell IL-4R α expression was required for optimal cytokine and antibody responses. Transfer of T- or B-cells from infected animals mediated IL-4R α dependent protection against primary infection independent of antibody. Analysis of cellular responses revealed roles for T-cell IL-4R α expression in the induction of effector T-cell populations and novel B-cell functions independent of antibody production. This study demonstrated haematopoietic expression of IL-4R α is essential for optimal immunity against parasitic nematodes. Furthermore disrupted IL-4R α expression on T- and B-cells severely impacted on the development of host immunity.

Pathogens, parasites, and the movement ecology of African water birds

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Animal populations are intricately connected to populations of their parasites and pathogens through a range of basic ecological variables, including such things as life history strategies, habitat use, host community composition, and dispersal. Spatial variation in host-parasite-pathogen relationships offers a valuable window into understanding the importance of these variables, particularly for wild animal populations. A two-year intensive field study was conducted to screen wild water bird communities in South Africa, Mozambique, Botswana, and Zimbabwe for avian influenza. A total of 4,977 birds of 165 species were sampled, from which 125 individual birds were influenza positive. Prevalence was highest at the Zimbabwe site, the adjacent Lakes Chivero and Manyame, and lowest at Strandfontein Watewater Treatment works near Cape Town. We also completed some 1,250 hours of standardized point counts at the study sites, providing a quantitative summary of the bird community. Overall avian influenza prevalence in the subregion appears to be around 2.5%, with considerable taxonomic variation, and there was no detectable influence of the seasonal arrival of palearctic migrants on regional avian influenza prevalence. Although published studies have largely focused on anatid ducks as reservoirs for avian influenza, we also found relatively high prevalence (5.5%) within the Dendrocygnidae (whistling ducks) and unexpectedly high prevalence in several passerine species. Influenza occurrences were highly variable within sites and between years. The results must be interpreted from within the context of the water bird community and their movement patterns and ecology. We have several years of data from satellite telemetry of 47 individuals of two common duck species, Red-billed Teal and Egyptian Geese; around 7 individuals of each species were tagged with satellite GPS transmitters at three different locations (Strandfontein, Barberspan, and Lake Manyame) along a c. 2,500km latitudinal gradient. I present a brief overview of some of our results from the tracking study and explain how our growing understanding of waterbird movement ecology should ultimately allow us to develop a stronger, more mechanistic understanding of the maintenance and spread of waterbird pathogens and parasites in southern Africa.

Patterns of parasite prevalence in farmed abalone (*Haliotis midae*) in South Africa

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Abalone are herbivorous marine gastropods with a wide distribution. People have consumed wild abalone since ancient times. Demand for abalone has led to the development of farms in many countries. South Africa is the largest producer of farmed abalone outside Asia. The industry has made use of health management services since 1998. Abalone are collected regularly from participating farms to evaluate their health, primarily by means of histology. As a result, farmed South African abalone are the most intensively surveyed abalone in the world. Data from the health program can be used to investigate the relationship between parasite prevalence in *Haliotis midae* and certain host and environmental factors. Previous work examined data collected for the period 1 January 2000 to 31 December 2004 from nine different abalone farms in the western Cape. Six types of parasites were identified, namely sessile peritrichous ciliates, renal coccidia, gut protozoa, digestive gland protozoa, rickettsia like prokaryotes and trematodes. The rarity of trematodes precludes meaningful analysis of their prevalence. It was shown that prevalence is associated with age for most parasites. Older animals had a higher prevalence of all parasites except for left kidney coccidian infections, which were more common in younger animals. Variations in parasite occurrence were seen for various localities, but this appeared unrelated to abalone densities. A significant association was found when investigating diet. Animals receiving kelp were more at risk of gut associated parasite infections than those on artificial feed. Finally, it was shown that animals in recirculating systems had a significantly lower prevalence of sessile ciliates, but more left kidney coccidian infections, than those in flow through. Continuation of the abalone health management program has led to a vastly enlarged data set and allowed confirmation of most of the findings of the original study. It has also been possible to identify temporal patterns in the occurrence of some parasites, notably increased prevalence of gut protozoa in winter. Data on stocking densities, flow rates and husbandry practices are available for the period 1 January 2009 to 31 December 2010 and the associations between these factors and parasite prevalence are explored. Possible applications of the results in controlling abalone diseases include implementation of well established biosecurity practices such as separation of age groups, rigorous culling of non performers, and minimising time to harvest. It is suggested that kelp should not be fed to younger abalone, as this increases the risk of gut associated parasite infection.

Epidemiology of *Trichinella* spp in Southern Africa – the missing links

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The first report of *Trichinella* species in muscles of farmed crocodiles (*Crocodylus niloticus*) was in 1995 by Foggin *et al.*, 1997. This was followed by experimental infections which confirmed that the *Trichinella* sp was infective to domestic pigs and laboratory mice. Morphological, biochemical and molecular studies confirmed that this was a new *Trichinella* sp and was designated *T. zimbabwensis*. The species infects mammals and reptiles excluding birds and the larvae are non-encapsulated in host muscles. This became the first report of a non-encapsulated *Trichinella* species in Africa and both adults and larvae are similar to *T. papuae*. In a 2002 survey conducted in Zimbabwe, 11(41%) of the 27 crocodile farms had infected animals. To date natural infections with the parasite have been reported in crocodiles (South Africa, Mozambique and Zimbabwe), varans (monitor lizards) (Zimbabwe) and lions (South Africa). The species was also confirmed to be a zoonosis as it was highly infective to captive baboons and monkeys. Several experimental studies have been conducted in various hosts with the aim of elucidating the epidemiology of the parasite in nature and fill-in the missing links. This presentation will highlight the current research areas which are pertinent in unlocking the missing links in the epidemiology of this parasite in nature and different hypotheses on the life cycle based on available information. The possible reasons of why trichinellosis is a neglected zoonosis in sub-Saharan Africa will also be presented.

Why is Southern African canine babesiosis so virulent? An evolutionary perspective

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Canine babesiosis is a common, highly virulent disease in Southern Africa with even pups and juveniles being severely affected. This contrasts with bovine babesiosis, for example, where host, parasite and vector co-evolved and young animals develop immunity after infection without showing clinical signs. *Babesia rossi*, the main causative organism of canine babesiosis in sub-Saharan Africa, was first described from a side-striped jackal *Canis adustus* in Kenya. Although data are meagre, there is evidence that indigenous African canids, such as jackals and wild dogs (*Lycaon pictus*), can harbour the parasite without showing untoward effects. Dogs are not indigenous to Africa. The vast majority of dogs presented at veterinary facilities in South Africa represent recently introduced European, Asian or American breeds. The contention is that *B. rossi* is a new challenge to which these dogs have not adapted. With intensive treatment of clinical cases, natural selection is effectively negated and the status quo will probably be maintained indefinitely. It is postulated that *Babesia vogeli*, which frequently results in unapparent infections or mild manifestations in dogs, represents or is closely related to the ancestral form of the canine parasite, possibly originating from wolves (*Canis lupus*).

Host specificity of fish parasites

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There are about 24 000 known fish species world wide of which about 40% are found in freshwater. They are the largest and oldest group of vertebrates on Earth and were already abundant during the Devonian, 400 million years ago. This long evolutionary history resulted in fish acquiring a large, diverse and mostly benign assembly of symbionts and parasites. These include representatives of different taxa ranging from Protozoa to Arthropoda and for the purpose of this paper are grouped according to their host associations. The first group includes symbionts and parasites restricted to a single host fish, endemic to a specific river drainage basin e.g. different species of the African endemic fish louse genus *Chonopeltis*. The second group has a narrow host range restricted to members of a specific fish family e.g. the copepod *Lamproglena monodi* which is host specific to a wide range of cichlid fishes in Malawi and the Zambezi system. The third group also has a narrow host range, but is associated with different non-related host species e.g. the fish louse *Dolops ranarum*, while the fourth group is opportunistic with a wide host range sometimes also found associated with other non-piscean hosts e.g. *Trichodina heterodentata* which infests many different fish species as well as tadpoles. The fifth group are alien introductions, via the introduction of fish. South Africa has a particularly bad record with alien introductions as 22 of our 98 freshwater fish species are aliens. Some alien parasites remain associated only with their alien host, such as *Trichodina acuta* from trout, but others spread to parasitise local species indiscriminately resulting in sporadic mass mortalities amongst cultured and wild fish populations. The introduction of the fish louse *Argulus japonicus*, the anchor worm (a copepod) *Lernaea cyprinacea* and the Asian tapeworm *Bothriocephalus acheilognathi* to South Africa and other parts of the world are linked to the distribution of different carp species. These three parasites have resulted in mass mortalities amongst wild local species as well as farmed carps in South Africa. They are now widespread throughout South Africa and mass mortalities of fishes often occur in impoundments in the Free State. These very same three parasites were also introduced to North America with similar consequences. The final group are species with ubiquitous distribution, they attack indiscriminately, are dangerous pathogens causing mortalities amongst cultured fish and are aquarists and the ornamental fish industry's worst nightmare. Amongst these, white spot disease, caused by the ciliophoran *Ichthyophthirius multifiliis*, chilodonellosis caused by two species of another cosmopolitan ciliophoran genus *Chilodonella* are major problems worldwide. Their origin and method of global dispersal is still unclear, and although introductions of alien fish species could also be responsible for their translocation there must be other, yet unknown, methods of dispersal as we also found them on wild fish in the Okavango Delta, a system so far unspoiled by alien fish introductions.

Oral presentations:

Avian malaria in Western Cape wetland passerines – prevalence in avian communities

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Despite the extensive global range of avian malaria, information remains scanty regarding the ecology of the disease in Africa and conditions favouring its transmission. In particular, there has not been much examination into transmission factors pertinent to host species community dynamics. Prior studies have demonstrated links between host and pathogen ecology, where host availability and/or host abundance have had an influence on pathogen prevalence within the host community. The potential existence of similar links between the ecology of the host and the prevalence or incidence of avian malaria, has not previously been explored. Findings on the ecology of similar vector-borne diseases indicate a possibility that avian malarial pathogens may also exhibit a preference for a particular host species. The present study aims to investigate avian malaria prevalence in passerine species from Western Cape wetlands. Passerines were sampled from 26 separate wetland sites found along the coastal gradient of the Western Cape. Point counts were conducted at each wetland site, which varied in bird species richness. To date, from 300 birds examined, 48 individuals (15%) from 13 different species have been found positive for avian malaria. Of the infected birds, 37 (77%) were found in birds from within the Ploceidae family. These results are presented with emphasis on the potential influence of factors pertinent to host community species composition and preferred host abundance.

Prevalence of vector-borne diseases in wild waterbird populations inhabiting six selected wetlands of the Western Cape Province of South Africa

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A survey was conducted to estimate the occurrence of vector-borne infections (malaria, West Nile virus and Newcastle disease virus) among wild birds (n = 200) inhabiting six selected wetlands of the Western Cape Province South Africa. The study mostly targeted ducks and waders. Avian malaria infection was evaluated by direct examination of blood smears. Haemosporidian parasites (Sporozoa: Haemosporida) were identified to species level following the guidelines of Valkiunas (2005). West Nile virus and Newcastle disease virus were detected using real-time polymerase chain reaction (RT-PCR) technique. Avian malaria parasite prevalence was generally low; only 1 of 55 Egyptian Goose trapped was infected with blood hematozoa. *Haemoproteus nettionis* (Haemosporida: Haemoproteidae) was the only avian haemosporidian parasite found in the blood of anatids sampled in this study. No infection was detected in the waders. Viral infections were equally low; 1 African Black Oystercatcher (*Haematopus moquini*) and 1 Yellow-billed duck (*Anas undulata*) tested positive for West Nile virus and Newcastle disease respectively. These findings suggest that avian malaria parasite prevalence in water birds is low and these parasites may not yet pose a serious threat to migrant and resident duck populations inhabiting wetlands in the Western Cape province of South Africa. However, the detection of West Nile virus in the endangered African Black Oystercatcher and presence of Newcastle disease virus in a Yellow billed duck at a dam located near poultry farms warrant further investigations.

Effects of climate and host life history traits on tick burdens in the Eastern rock elephant shrew (*Elephantulus myurus*)

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The distribution of parasites is often characterized by substantial aggregation with a small proportion of hosts harbouring the majority of parasites. This pattern can be generated by abiotic and biotic factors that affect both parasites and their hosts and determine host exposure and susceptibility to parasites. Climate factors can influence both, parasite survival and a host's investment in life-history traits, generating temporal patterns of parasite aggregation. In addition, host life-history traits such as sex and age determine resource allocation in competing functions such as growth, reproduction and parasite defence. Consequently, host factors may further modify parasite aggregation patterns among hosts. We investigated the effects of climate factors and life-history traits on tick loads of the Eastern rock elephant shrew or sengi (*Elephantulus myurus*). A total of 113 elephant shrews were captured throughout an entire calendar year and climate data were collected during the study period. All individuals harboured large numbers of the immature stages of a single tick species (*Rhipicephalus* near *warburtoni*). Monthly tick burdens varied significantly for both larvae and nymphs and varied with the amount of precipitation two months prior to the capture month for both stages. At the same time, larval tick burdens decreased with increasing maximal ambient temperatures while nymphal burdens decreased with increasing minimal temperatures. Whereas the former is probably a consequence of an increased desiccation risk the latter is likely the result of increased feeding durations due to extended torpor bouts of the host during cold periods. No effect of sex on tick burdens was apparent for monthly counts. However, when considering breeding vs. non-breeding season and taking host age into account adult males harboured the greatest larval tick burdens during the breeding season. In contrast neither sex nor age affected larval tick abundances during the non-breeding season. Our results suggest that climate factors largely constraint the most susceptible tick stage (i.e. larvae) while otherwise host-linked factors play a major role in generating aggregation patterns of *R. near warburtoni*.

Effects of seasonality and host life history characteristics on the prevalence and abundance of ectoparasites in the Namaqua rock mouse (*Micaelamys namaquensis*)

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The distribution of ectoparasites within its host population is usually affected by various factors such as seasonality and host gender. The present study aimed to investigate the variation of parasitic infestations and the parasite community that inhabits the Namaqua rock mouse (*Micaelamys namaquensis*) (Smith, 1834). The Namaqua rock mouse has an ubiquitous distribution in southern Africa and may accordingly be exposed to a large diversity of ectoparasites. During the period April 2010-January 2011, a total of 264 of the Namaqua rock mice were live-trapped and examined for ectoparasites in Ezemvelo Nature Reserve, Gauteng Province, South Africa. A total of 13 species of ectoparasites were recovered from these animals. The collection comprised of three species of fleas (*Epirimia aganippes*, *Praopssylla powelli*, *Chiastopssylla pitchfordi*), five species of mites (*Androlaelaps marshalli*, *Androlaelaps rhapsomysi*, *Laelaps* sp. I, *Laelaps* sp. II, *Trombiculidae*) and five species of ticks (*Rhipiciphalus appendiculatus*, *R. pravus*, *R. simus*, *Hyalomma rufipes*, *Haemaphysalis elliptica*). Our results showed that the prevalence and abundance of ectoparasites varied seasonally, however, the trends in seasonal patterns differed between different parasite groups (i.e. fleas, mites and ticks). This seasonal variation in infestation patterns is likely to be linked to the varying life-cycles of the parasite groups. Similarly, sex-biases in parasite loads were observed for some ectoparasite groups but not others suggesting that host life-history traits contribute to infestation patterns. Our data implicates Namaqua rock mice as hosts for economically important ectoparasite species such as *R. appendiculatus* a known vector of several livestock diseases such as the East Coast fever in cattle. Further exploration of the host-parasite dynamics of Namaqua rock mice may provide key information for management policies of such diseases in livestock.

The effect of anthropogenic habitat change on the gastrointestinal parasite burden of the striped mouse (*Rhabdomys pumilio*) in the Western Cape Province

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Parasites are omnipresent in the lives of wild animals and represent a major component of biological diversity. More than 50% of the known species on this planet are parasites or pathogens of some form. In southern Africa there is a paucity of information on small mammal parasite ecology and how the parasite assemblage is affected by habitat transformation. During the process of landscape transformation and urbanization new environments are created, where both animals and humans are exposed to new parasites and the opportunity exists for parasite exchange. As a consequence it is hypothesized that host-switching events and the possibility of emerging infectious diseases will be higher in transformed habitats. Using the four-striped mouse *Rhabdomys pumilio* as a model entity, the objective of the project is to investigate the effect of habitat change on the helminth diversity and species assemblage of an agriculturally adapted small mammal species. Sampling took place at four localities in the Western Cape Province within each of four well characterized habitat types: A - pristine natural, B - agricultural (crops and vineyards), C - agricultural (life stock) and D - urban areas. More than 500 (n=521) mice have been collected. So far a total of 167233 helminths were recorded and seven helminth species have been identified. The nematode *Heligmonina spira* was with 92.2% the most abundant worm. In addition two cestode and one trematode species were recovered. Preliminary results suggest that conditions associated with agricultural activities facilitate a higher mean abundance of individual helminths. Furthermore a positive body size relationship was found between host size and helminth infestation. Ongoing data analyses of the still outstanding samples will highlight the importance of agricultural and urban activities in shaping natural parasite assemblages and results will be presented in this talk.

The kangaroo leech *Marsupiobdella africana*: an integrative model for the evolution of reproduction and transmission strategies

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It has been well established that transmission opportunities are the main forces shaping the diversity of parasitic life-history traits and their related reproduction strategies. Consequently the evolution of parasitic life cycles has been considered in the light of enhancers and/or constraints on parasitic survival depending on the availability of environmental resources. However, several transmission patterns observed among leeches (Hirudinea) occasionally differ from the clear-cut definitions commonly used in parasitology and lead to a different understanding of host-parasite-environment interactions. A range of reproductive behaviors has been described for leeches, including anything from the direct deposition of cocoons on the substrate to their incubation on the ventral surface of the parent. *Marsupiobdella africana*, parasite of *Xenopus laevis*, displays the most extreme parental care strategy. As a convergence to the kangaroo feature, a brood pouch on the ventral surface ensures protected development of the offspring, as well as increasing the likelihood of encountering the host. To date, a single study investigated the ecology of this species and reported that while feeding activity takes place on the frog, mating only occurs after detachment from the host. However, sexually mature and egg-carrying individuals recovered from the freshwater crab *Potamonautes* sp. revealed a surprising and undefined role in the process of transmission. Because of this highly derived reproduction strategy and the implication of an unexpected host in the life cycle, we undertook a combined study of the ecology of *M. africana* through field surveys and experimental approaches. The dynamics of infestation in the field has been followed through mark and recapture of the two host species. Several hypotheses on the behavior and survival rates of leeches were also tested under lab conditions to evaluate the nature of the relationship between the three partners in the system. The study of this promising model reveals key aspects of host-parasite-environment interactions. First, the reproductive strategy that would arise from selective pressures on individual survival involves direct draw backs on the transmission patterns, which in turn are balanced by changes in species ecology. Second, the role and importance of an intermediate host has to be considered not only from a development or transmission point of view, but also in terms of the broader parasitic species ecology perspective.

Parasite-mediated female mate choice in highveld mole-rats (*Cryptomys hottentotus pretoriae*)

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Animals have evolved behavioural mechanisms to recognize and avoid infected conspecifics. In mammals social interactions are often governed by olfactory cues and they have been found to convey a multitude of information such as the sex and infection status of an individual. Olfactory cues play a pivotal role in mate choice in mammals and it has been suggested that they function as sexual courtship displays. As such the expression of olfactory signals has been linked to male testosterone levels. Evidence suggests that males exhibit lower testosterone values during infection while corticosteroid levels can be elevated and this may render their scent less attractive to females. However, scent preferences are not only determined by the senders' condition but also by that of the receiver and preferences have been shown to be variable depending on the infection status of the individual tested. We evaluated these hypotheses in highveld mole-rats, *Cryptomys hottentotus pretoriae* by allowing females with different infestation status to explore a Y-maze containing odour samples from males either infested or uninfested with the cestode *Mathevotaenia* sp. We measured the levels of testosterone and cortisol in male samples as well as the exploration behaviour of females. Our results do not show an effect of infestation status on male testosterone or cortisol levels. The two female groups exhibited distinct differences in their behaviour and while uninfested females spend more time with the odour sample of infested males the opposite was true for infested females. This was partially a result of the significantly lower overall activity of infested females. These females also tended to be lighter than healthy females suggesting that their ability to choose may have been impaired. Interestingly, there was no indication that testosterone levels played a role in mate choice behaviour. Conversely, the odour preferences of uninfested females were linked to cortisol levels of male odour. The results suggest that parasites may impair the ability to choose a mate in female highveld mole-rats and that this may be linked to energetic constraints as well as sensory modifications.

Comparison of the prevalence and intensity of infestation of selected ectoparasites of small mammals at Avis Dam, Windhoek, Namibia, using non-destructive and destructive collection techniques

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Most studies on fleas, ticks, lice and mites of small mammals involve killing the hosts in order to collect the ectoparasites. In this paper, we present preliminary results of a study conducted at Avis dam in the city of Windhoek in order to test the null hypothesis that there would be no significant difference in the prevalence and intensity of infestation of ectoparasites (fleas, ticks, lice and mites) of small mammals using destructive (necropsy) and non-destructive (live-collection) collection techniques. Small mammals (rodents and shrews) were live-trapped using Sherman traps. All captured small mammals were restrained in a transparent Perspex tube and brushed thoroughly to dislodge and remove ectoparasites. Each live-brushed small mammal was subsequently necropsied and brushed further to collect any remaining ectoparasites. Fleas were processed using standard parasitological procedures and were mounted permanently onto slides. The total number of fleas, ticks, lice and mites collected during the live-brushing and the necropsy were added up to give the total numbers for the destructive collection technique for each small mammal host. Fleas and ticks were identified to species level. A total of 34 small mammals belonging to 6 species i.e. *Elephantulus intufi*, *Gerbilliscus leucogaster*, *Mastomys spp*, *Gerbillurus paeba*, *Rhabdomys pumilio* and *Thallomys nigricauda* were captured. There was no significant difference in the prevalence and intensity of infestation of fleas, ticks and mites of small mammals as determined by destructive and non-destructive collection techniques. However, the prevalence of infestation of lice on small mammals was higher for destructive (41%) than non-destructive (12%) collection techniques. The paper discusses the conservation benefits of employing non-destructive collection techniques for studies on ectoparasites of small mammals.

The micromorphology of an undescribed *Colpocephalum* louse from the Cape vulture

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Colpocephalum lice are obligate ectoparasites which belong to the Menoponidae, with *Colpocephalum turbinatum* reported on several indigenous vulture species. These feather lice are found on the fine plumage where they feed on the epidermal debris and fine barbules resulting in damage to these feathers. As a number of these vulture species are ecologically threatened, it is important to investigate how these ectoparasites impact their hosts. *Colpocephalum* lice were one of three lice species collected from Cape vultures during a ringing exercise at Blouberg nature reserve in Limpopo. Laboratory studies revealed that these *Colpocephalum* lice differed greatly from the expected *C. turbinatum* species reported from the Cape vulture (*Gyps coprotheres*). This study aimed at investigating the taxonomic micromorphology for identification or description of this species. Lice were collected and fixed directly in 70% ethanol, routinely prepared for SEM and viewed in a Leica Stereoscan 420 scanning electron microscope at 5 – 10 kV. The studies revealed a louse with a large shovel-shaped Menopon head with preocular slits and setation typical of *Colpocephalum* lice. The oral cavity, mouthparts, and a pair of small labial palps with five anterior setae were anterioventrally situated. The four-segmented maxillary palps were directed anteriolaterally to the mouthparts and bore 14 terminal sensilla. The antennae were pedunculate in form and protectively carried in deep antennal grooves laterally on the head which is characteristic of the suborder Amblycera. The pro-, meso- and metathorax were easily identifiable ventrally. The prothorax bore a unique hooked process on the ventral plate. The three pairs of robust legs shared common characteristics such as the pad-like lobe the euplantula on the distal end of each tarsus and the pair of curved pretarsal claws. However, each leg also had distinctive characteristics, with legs I having a second pad-like lobe not mentioned in the literature on the pretarsus just proximal to the claws. These lobes are not shown in the descriptions of *C. turbinatum*. Legs II and III lack these pretarsal lobes. Legs III carries four ctenidia consisting of comblike rows of setae on the ventral femur which are characteristic in the *Colpocephalum* complex. Only three ctenidia are indicated for *C. turbinatum*. The abdomen was distinctively rounded in shape with the segments I to VIII having well-developed lateral paratergites. Tergites VIII and IX are fused in an arch-shape plate while sternites VIII and IX are fused forming two large subgenital plates which extended posteriorly beyond the genital opening. Spiracles open through oval spiracular plates on sternites III to VIII while the postspiracular setae were arranged on the paratergites. Another characteristic was the arrangement of double rows of ctenidia on the lateral edges of sternites II to IV. The SEM greatly enhanced the study of these taxomorphological specializations of this species of *Colpocephalum* lice.

Biological and behavioural characterization of *Anopheles funestus*-like species from Northern Malawi

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Malaria in Malawi is transmitted by *An. gambiae*, *An. arabiensis* and *An. funestus*. This vector diversity is further complicated by the recently discovered new *An. funestus*-like species which is morphologically similar to *An. funestus*, is found sympatrically and in association with humans. The objective of this study was to investigate the biology and behavior of the *An. funestus*-like species and its possible role in malaria transmission. *Anopheles* mosquitoes were collected resting indoors and outdoors from Karonga and Likoma Island in Malawi. Specimens were identified morphologically as belonging to the *An. funestus* group and further by PCR as *An. funestus*-like species. Identified samples were analyzed by ELISA for blood meal source and *Plasmodium* sporozoite infection. A total of 392 adult *An. funestus* group mosquitoes were collected during April and September, 2010. Karonga contributed for 63.8% and Likoma Island 36.2%. Of the total, *An. funestus*-like comprised 8.7% (34/392). Most of the *An. funestus*-like species were collected resting indoors 91.2% (31/34) compared to outdoors 8.8% (3/34). The species was predominant in the dry season 64.7% (22/34). Nineteen *An. funestus*-like females were analyzed for blood meal source. Goat blood was found in 10 specimens, of which 7 had also taken cow blood. An overall dry season infection rate of *An. funestus*-like species by *Plasmodium vivax* was 5% (1/20) in this study and 3.1% (2/64) from a 2009 analysis. This study confirmed the existence of *An. funestus*-like species occurring in sympatry with *An. funestus* group members. *An. funestus*-like species was predominantly found resting indoors (endophilic) but preferring animal over human blood (zoophilic). This species appears to be a vector of *P. vivax* and not *P. falciparum*.

Repellency and toxicity of essential oils of *Mentha piperita* and *Mentha spicata* on larvae and adults of *Amblyomma hebraeum* (Acari: Ixodidae)

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The toxicity and repellency effects of essential oils of *M. piperita* and *M. spicata* at concentrations of 5%, 10% and 20% v/v were evaluated against adults of *Amblyomma hebraeum* using fumigant toxicity and glass plate repellency bioassays. The feeding deterrent of essential oils of *M. piperita* and *M. spicata* on larvae of *Amblyomma hebraeum* was also tested using feeding deterrent bioassay. High percentage repellency (range 90 -100) was observed at all concentrations of both essential oils of *M. piperita* and *M. spicata*. The repellency for 5%, 10% and 20% v/v concentrations of essential oil of *M. piperita* persisted for 60, 40 and 20 minutes respectively and eventually the ticks died during the experiment. While, the repellency of essential oil of *M. spicata* persisted for 80, 50 and 30 minutes at 5%, 10% and 20% v/v respectively and the ticks also died thereafter. The mean mortality effects of the essential oils of *M. piperita* and *M. spicata* at all concentrations was 100% at different time intervals. Larvae treated with both essential oils did not attach and engorge on rabbits. GC-MS analysis of both essential oils showed significant variations in the concentration of active compounds such as terpenene, menthol and piperitone (P<0.01).

The functional micromorphology of the louse *Falcolipeurus quadripustulatus* from the Cape vulture

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Falcolipeurus quadripustulatus are obligate ectoparasites which belongs to the Philopteridae, found on several vulture species. These specialist feather lice are found on the plumage, usually firmly attached by the mandibles, although they may move sideways across the coverts with amazing rapidity. As specialist ectoparasites they feed on the epidermal debris and snippets of barbs and barbules resulting in significant damage to the feathers. As a number of these vulture species are ecologically threatened, it is important to investigate how these ectoparasites impact their hosts. *F. quadripustulatus* was one of three lice species collected from Cape vultures (*Gyps coprotheres*) during a ringing exercise at Blouberg nature reserve in Limpopo. This study aimed at investigating the functional micromorphology and to obtain some understanding of how these lice attach to, orientate and feed on their hosts. Lice were collected and fixed directly in 70% ethanol, routinely prepared for SEM and viewed in a Leica Stereoscan 420 scanning electron microscope at 5 – 10 kV. The studies revealed a long, slender louse that was dorso-ventrally flattened to fit into the grooves between the feather barbs. It had a primitive ischnoceran head, with the oral cavity and mouthparts situated ventrally in a distinctive clypeal groove. The transversely held mandibles rotated on prominent external condyles and were distally deeply notched to grasp and shear off pieces of the feather barbules for ingestion. Posterior to the mandibles was the labrum, bearing a pair of labial palps terminating in six sensory setae. The sexually dimorphic antennae had five segments in both sexes, and was filiform in the female. The scape was enlarged and robust in the male, with a prominent grooved thumb-like process on the posterior margin, while the flagellomere I formed a long curved hook. These hooked processes are used for attachment to the female during copulation. The antenna terminated in a peg organ with 11 sensilla. A plate and a pore organ were visible on both of the two distal flagellomeres. These sensory organs are used to locate attachment sites, prospective mates and egg-laying sites on the feathers. The pterothoracic and abdominal segments showed distinctive patterns of micro-ornamentation. Round open spiracles were observed on abdominal segments III to VIII. Internally the spiracular openings were lined by an irregular honeycomb network which could function to filter air entering the trachea. Each of the legs terminated in 2 long, curved claws with the ability to lock over barbules between three opposing setae on the pretarsal sclerites. A row of 6 short, robust spines was distinct on each elongated tibia and extended as a row of 8 spines on each elongated femur. This study revealed two latero-ventral hooks on the terminal male genital segments. A unique heartshaped plate with 8 distal setae was observed on the ventral surface of the terminal genital segments of the female. These terminal specializations may indicate a 'locking device' used during copulation. The SEM greatly enhanced the study of the micromorphology of these specializations of *F. quadripustulatus*.

Effects of aquaculture return flows on parasite assemblages of *Clinus superciliosus*

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A mariculture farm situated at Jacobsbaai on the west coast of South Africa, discharges its wastewater into a nearby bay, possibly resulting in increased nutrient levels localised in this bay. This study aimed to determine the influence of nutrient input on fish parasite assemblages and use this to infer the health of the ecosystem. The use of fish parasites as indicators of ecosystem health is a relatively new idea, recently implemented in other parts of the world. Thirty fish (*Clinus superciliosus*) were caught at both the impacted bay and a control site in Jacobsbaai. Fish were weighed, measured and dissected and parasites from the skin, gills, intestine, blood and bile identified and counted. Fish from the impacted site were in better condition, with significantly higher average weight and length values ($t=-3.29$, $p<0.01$ and $t=-3.32$, $p<0.01$ respectively) than at the control site, possibly due to increased food availability at this site due to nutrient input. Fish from the impacted site had a significantly higher abundance of parasites than fish from the reference site ($t=-2.32$, $p<0.05$). This was likely due to the greater length of the fish at this site, as determined by a general linear model ($F= 17.15$, $p<0.01$), allowing more parasites to proliferate on fish. Through the use of multivariate analyses it was found that the fish at the impacted site housed significantly different parasite assemblages to those at the reference site ($R= 0.272$, $p<0.01$). It is concluded that the level of eutrophication at the impacted site was not sufficient to cause stress in the fish hosts, reduce ectoparasites or eliminate intermediate hosts; rather increased nutrient levels increased productivity allowing multiple-host life-cycle parasites to proliferate.

Towards addressing the current state of confusion within the Hexabothriidae Price 1942: *Callorhynchocotyle*, *Suriano* and *Incorvaia* 1982 (Monogenea: Hexabothriidae) re-visited with the preliminary evaluation of novel parameters for measuring haptoral armature of hexabothriids

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Representatives of Hexabothriidae Price, 1942 are oligonchoinean monogeneans, exclusively parasitic on the gills of chondrichthyan host fishes. Fifteen genera are currently recognised as valid, including *Hexabothrium* Nordmann, 1840; *Erpocotyle* van Beneden and Hesse, 1863; *Rajonchocotyle* Cerfontaine, 1899; *Squalonchocotyle* Cerfontaine, 1899; *Heteronchocotyle* Brooks, 1934; *Pseudohexabothrium* Brinkmann, 1952; *Rhinobatonchocotyle* Doran, 1953; *Dasyonchocotyle* Hargis, 1955; *Neonchocotyle* Ktari and Maillard, 1972; *Epicotyle* Euzet and Maillard, 1974; *Protocotyle* Euzet and Maillard, 1974; *Pristonchocotyle* Watson and Thorson, 1976, *Paraheteronchocotyle* Mayes, Brooks and Thorson, 1981, *Callorhynchocotyle* *Suriano* and *Incorvaia*, 1982, and *Branchotenthes* Bullard and Dippenaar, 2003. Although the hexabothriid literature is historically plagued with inconsistencies part of the recent confusion within the family follows the most recent revision of the family. Most authors on hexabothriid taxa agree over the importance of the haptoral sclerites in discriminating between species albeit with a lack of consensus on how this should be done. This lack of consensus adds to further confusion in the literature. The lack of consensus for a single accepted measurement protocol for the sucker complex sclerites and hamuli in Hexabothriidae supports the call for a standard which will assist in providing a level of stability. This process is as controversial as it is necessary. As such, we report here the preliminary findings of the investigation of a new measurement protocol for haptoral armature in the hope that it fuels a constructive debate around the current problems inhibiting progress in the Hexabothriidae. This protocol is tested herein on members of *Callorhynchocotyle* because of the errors requiring attention that are evident in much of the *Callorhynchocotyle* literature, and because all 5 species were relatively easily accessible either through the direct collection of new voucher material or through existing museum collections. However, it should be noted that the resolution of the statistical analyses is currently restricted in some cases by sample size, and the addition of larger sample sizes in future may well provide a higher level of resolution through the reduction of variance.

A short history of abalone tubercle mycosis in South Africa

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Abalone tubercle mycosis, an emergent fungal disease, caused by the peronosporomycete, *Haliotidica noduliformans*, was reported from abalone production facilities for the first time in October 2006. A targeted surveillance program for abalone tubercle mycosis was initiated for affected farms as well as any additional farms that were considered to be at risk. Since higher prevalence of abalone tubercle mycosis was measured on farms using recirculation systems, these sites and those within the proximity of the confirmed infected farms were considered to be at risk. Affected farms typically have high mortality levels, with up to 90% mortality in spat and up to 30% mortality in older animals. For ongoing surveillance purposes, stock assessments have been extended to all aquaculture permit holders and the degree of mortality is used as an indicator in the stock inspection process and is also used to estimate facilities at risk of infectious disease in the absence of confirmatory diagnosis. Abalone tubercle mycosis is currently identified by visual inspection and histological examination is used to confirm the presence of fungal hyphae. Since these diagnostic methods are accurate only when animals are exhibiting clinical signs, accuracy of diagnoses of animals not exhibiting clinical signs remains in question and may thus further elevate the risks posed by this pathogen. Subsequent identification and characterization of the fungus responsible for abalone tubercle mycosis in South Africa has greatly improved management and containment of this pathogen. The pathogen is currently managed as a production type disease and effective containment has been achieved by destocking of affected raceways/ facilities, sterilisation of equipment, removal and decontamination of biological filter material (when present) and adherence to suitable fallowing periods. Collectively, these measures have successfully mitigated the risk for the release, exposure and establishment of this pathogen in South Africa.

Helminth larval forms from freshwater fishes of a South African impoundment

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Parasites are of economic importance in both natural waters and more so in aquaculture. The starting point is usually their identification, then their ecology and lastly prevention and control. This study is part of a larger project on the helminth parasites of freshwater fishes and addresses the identification and ecological statistics of larval forms that use fish as intermediate hosts and birds as final hosts. Lake Tzaneen forms the major part of the Tzaneen Dam Nature Reserve with multitude activities that include fishing and angling, with fish farming and conservation strategies in the planning stages. During the routine examination of hosts for parasites, metacercariae were procured from the brain, branchial, visceral and heart cavities as well as the eyes. Metacercariae were killed and fixed in hot (+70 °C) alcohol-formal-acetate whilst nematodes were fixed using glacial acetic acid. The specimens were directly transferred into 70% ethanol for storage. The standard procedure for staining was followed and this comprised rehydration, staining (using aceto alum carmine solution), dehydration and clearing with clove oil. Temporary mounts were done on microscopic slides and the worms were identified using a BX51 clinical microscope through morphological analyses, photographs, drawings, dimensions and literature. Diplostomulum were obtained from *C. gariepinus*, *L. marequensis*, *O. mossambicus* and *C. flaviventris* with prevalence of 8% and mean intensity of 16. *Clinostomum metacercariae* had prevalence of 33% in *O. mossambicus* and a prevalence of 5% in *S. intermedius*. The prevalence for *Ligula intestinalis* in both *B. radiatus* and *B. unitaeniatus* was 100%, in *M. brevianalis* was 7%, in *M. salmoides* was 6% and in *L. marequensis* was 3%. All the five host species had mean intensity of 1 each. The gryporhynchid larvae had prevalence of 31% and mean intensity of 29 in *O. mossambicus*, and prevalence of 5% and mean intensity of 1 in *T. rendalli*. Contracaecum larvae had the following statistics: prevalence of 49% and mean intensity of 20 (*C. gariepinus*), 38% and 10 (*S. intermedius*), 19% and 4 (*O. mossambicus*), 10% and 3 (*B. trimaculatus*) 10% and 1.5 (*T. rendalli*) and 3% and 2 (*M. salmoides*). There were also numerous small unidentified cysts that were lodged in the skin (black spots), the gills and the visceral cavity. Though the life cycles of all these larvae are indirect and not troublesome in fish farming and aquaculture, the effects of their epizootiology and pathology cannot be underestimated.

Invasive threat: Parasites from red-eared sliders now infecting native terrapins in France and South Africa

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Terrapins are popular in the pet and food trade. Especially juvenile Red Eared Sliders are attractive but tend to lose their colours as they grow. This results in them being released into the environment where they establish natural populations due to their ability to adapt to a broad range of habitats. It's estimated that between 1988 and 1994 roughly 26 million *Trachemys scripta elegans* specimens were exported worldwide. Some countries banned turtle imports. Nevertheless, 3-4 million terrapin hatchlings are still being exported each year. Terrapins of the genera *Chrysemys*, *Graptemys*, *Trachemys* and *Pseudemys* were imported into France as pets and many were released. *Trachemys* became a invasive threat to indigenous species, *Emys orbicularis* in some habitats, as they compete for resources because *Trachemys* is bigger and more aggressive. The same happened in South Africa where feral populations of *Trachemys* were documented near Durban and Pretoria and probably contributed to the local extinction of *Pelusios rhodesianus*. A wide variety of small little Terrapins are infected with various parasites. These parasites are transported with the terrapin wherever it goes. When terrapins are released and natural populations are established, their parasites can escape and switch hosts to infect native species. Evolutionary old parasitic relations rarely have unfavorable effects on the host, but when host switching take place and new parasitic relations are formed, infections often are relentless with unfavorable effects. Three Monogenetic flatworms are known from terrapins and are found on most continents. This study will determine the extent to which invasive blood and flatworm parasites spread from invasive American freshwater terrapins to native species in France and South Africa and determine the conservation threats posed by them. Recently we discovered polystome infections in both *Trachemys* and *Pelomedusa* in South Africa.

Ectoparasites of *Schilbe intermedius* from five water bodies in the Limpopo Province of South Africa

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The present study deals with the survey of ectoparasites from *Schilbe intermedius* (Silver catfish). Parasitological surveys were carried out at five different localities in the Limpopo Province of South Africa. The Nwanedi-Luphephe Dam and Nandoni Dam, both part of the Limpopo River System, and Flag Boshielo Dam, Phalaborwa Barrage and Tzaneen Dam forming part of the Olifants River System. Seasonal surveys were done between 2009 and 2011. The hosts (Nwanedi-Luphephe Dam, n = 60; Nandoni Dam, n = 89; Tzaneen Dam, n = 92; Flag Boshielo Dam, n = 65; Phalaborwa Barrage, n = 60) were collected using gill nets of different mesh sizes. As soon as the fish were removed from the nets, macroscopic examinations were done on the boat for mobile ectoparasites. Skin smears were made and scrutinized for parasites. Each fish was weighed, measured and then sacrificed by neural pithing. Gills were removed and inspected for parasites with the aid of a stereomicroscope. Monogeneans were removed and mounted on slides using glycerine jelly. Parasitic crustaceans were preserved in 70% ethanol. Two genera of monogeneans that include 8 species are described from *S. intermedius* from African freshwaters, but there are no records for South Africa. The following parasites were recorded during this study: three monogenean species of the genus *Schilbetrema* from the gills; one species of branchiurian (*Dolops ranarum*) from the skin; and one copepod species (*Ergasilus* sp.) from the gills. *Schilbetrema quadricornis* was found to be the dominant monogenean and recorded from all five sites. The prevalence of this parasite was high (>80%) at all localities, except for Tzaneen Dam where the prevalence was notably lower (46%). The prevalence of *S. acornis* varied between the localities, and this parasite was absent from Nandoni and Tzaneen Dams. *Schilbetrema undinula* were only recorded from the Phalaborwa Barrage. Some of the hosts were highly infected with *Schilbetrema* spp. (>300) and might have a negative effect on the condition of those specific hosts. *Dolops ranarum* was absent from the Olifants River sites (Flag Boshielo Dam, Phalaborwa Barrage and Tzaneen Dam). *Ergasilus* sp. was recorded from two hosts only, both collected at the Phalaborwa Barrage during the summer of 2010.

Seasonal dynamics and occurrence of metazoan parasites of three cyprinids at the Nwanedi-Luphephe Dams in the Limpopo Province, South Africa

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The aim of this study was to provide information about seasonal variations and also report on certain ecological aspects of both ecto- and endoparasites infecting three cyprinids at the Nwanedi-Luphephe Dams (22°39'S 30°25'E), Limpopo River System. *Labeobarbus marequensis* (n = 53), *Barbus trimaculatus* (n = 63) and *Barbus radiatus* (n = 46) were sampled by means of gill nets and electrofishing or seine netting in accordance with the habitat conditions. The following dactylogyrids were found: *Dactylogyrus spinicirrus* was recovered from all hosts, *Dactylogyrus afrolongicornis alberti* and *Dactylogyrus afrolongicornis afrolongicornis* were both recovered from *B. trimaculatus*. Endoparasites recorded included the following groups: digeneans – *Diplostomulum metacercariae* from the eyes of *Lb. marequensis* and *B. trimaculatus*; *Ornithodiplostomum* sp. and *Neascus* sp. from both *B. trimaculatus* and *B. radiatus*. Unidentified digenean cysts were recovered from the gills and in the body cavity of both *Lb. marequensis* and *B. trimaculatus*; nematodes were represented by *Contracaecum* larvae in the body cavity of both *Lb. marequensis* and *B. trimaculatus* while cestodes were represented by gryporynchid larvae from the intestine of *B. radiatus*. The intensity of the parasites varied amongst the different seasons and hosts. Summer and spring appeared to be optimal seasons for *D. spinicirrus*. There was no correlation between either fish length or condition factor and the number of parasites. The study indicated that the abundance of monogeneans was partially influenced by seasonal change.

Treating monogeneans in aquaculture in South Africa: Using “off-label” Praziquantel formulations as bath treatments for *Diplectanum oliveri* in dusky kob culture systems

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Due to the limited number and availability of registered or approved chemotherapeutants for the treatment of diseases in marine finfish aquaculture facilities in South Africa, an immediate short term option to evaluate the efficacy of an “off-label” drug as a treatment solution for *Diplectanum oliveri* infections on dusky kob was pursued. A commercial anthelmintic, currently registered for use in terrestrial herbivores for the treatment of liver “flukes” (*Fasciola* spp.), containing praziquantel as the active ingredient was tested and proved to be effective. The efficacy of the two formulations of this anthelmintic, solution and suspension, were compared in their ability to reduce these parasites on the gills of the fish. The effect of the drugs on parasite population dynamics, changes in habitus of fish and stress induced by the treatments on the fish were also determined. Four treatment combinations for each formulation were evaluated; short exposure/low concentration (2 hours/2 ppm), short exposure/high concentration (2 hours/20ppm), long exposure/low concentration (24 hours/2ppm) and long exposure/high concentration (24 hours/20ppm). The high concentration treatments eliminated all adult parasites, but caused a significant stress response by the host fish. Conversely, the low concentrations failed to remove all adult parasites.

Metazoan parasites of the Mozambique tilapia from different localities – can water quality clarify the presence or absence of parasites?

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Seasonal parasitological surveys were conducted from June 2009 to April 2010 at Flag Boshielo Dam (FBD) and Loskop Dam (October 2010) as part of a larger project on the health of *Oreochromis mossambicus* (Mozambique tilapia) in the Olifants River System. Monogeneans were mounted and cleared in either ammonium picrate solution or glycerine jelly. All other parasites were fixed and preserved in 70% ethanol. Water samples were taken concurrently and selected variables determined. The parasite assemblage comprised 17 species in total. The following parasites were recorded from FBD (mean abundance indicated): four *Cichlidogyrus* spp. (3.83); three *Enterogyrus* spp. (2.3); *Clinostomum* sp. (0.03); digenean larvae from gills (0.10); digenean larvae from the skin (0.03); gyporhynchid larvae (0.23); *Contracaecum* larvae (0.23) and *Ergasilus* sp. (0.10). The following parasites were recorded at Loskop Dam: *Cichlidogyrus* spp. (1.75); *Gyrodactylus* sp. (0.06); digenean larvae from the skin (0.13); gyporhynchid larvae (1.25); *Contracaecum* larvae (0.88); *Ergasilus* sp. (0.31); *Lernaea* sp. (0.06) and *Argulus* sp. (0.25). The water quality was generally poorer at Loskop Dam than at Flag Boshielo Dam. The monogeneans had a higher abundance at the site with better water quality while the abundance of the endoparasites was generally higher at Loskop Dam (with *Clinostomum* sp. the exception). But, three ectoparasite species, i.e. *Gyrodactylus* sp., *Lernaea* sp. and *Argulus* sp., were only recorded from Loskop Dam, the site with the poorer water quality. The highest number of a parasite species found per fish was 38 at Flag Boshielo Dam, but six at Loskop Dam. The variability of the calculated biotic indices and the degree of interactivity among parasites suggests that the structure of parasite communities (especially the different groups, i.e. monogeneans, digeneans, cestodes, nematodes, copepods and branchiurans), is affected differently by water contamination levels and the type of pollution (e.g. organic or metal pollution).

***Pseudoleptobothrium christisoni* n. sp. (Monogenea: Microbothriidae) off the dermal denticles of *Rhinobatos annulatus* (Rhinobatidae) from the Southern tip of Africa**

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Pseudoleptobothrium christisoni n. sp. is described from the dermal denticles of the dorsal skin surface of a single female *Rhinobatos annulatus* collected off Cape Agulhas, South Africa and destined for public exhibition at the Two Oceans Aquarium in Cape Town. This new species differs from the only other species in the genus, *P. aptychotremae* Young, 1967, primarily by the morphology of the male copulatory tract. The distal portion of the male copulatory tract is wide and covered with muscular papillae. In addition, the area of ventral tegument near the vaginal pore has several parallel ridges which appear to serve as initial attachment for a bipartite spermatophore. The formation of the spermatophore in *P. christisoni* is discussed. The generic diagnosis of *Pseudoleptobothrium* is revised to accommodate *P. christisoni* and a partial redescription of *P. aptychotremae* is provided to include characters previously not described or described incorrectly.

Detection of *Babesia rossi* genotypes using real-time PCR assay

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Currently three species of large *Babesias* are known to infect dogs, namely *Babesia canis*, *Babesia vogeli* and *Babesia rossi*. The microscopic identification of large piroplasms in canine erythrocytes was sufficient for the diagnosis of *Babesia canis* infection. Geographic location, differences in pathogenicity, antigenic variation, and differences in vector specificity have all been used to support the existence of three subspecies. In this study we used real-time polymerase chain reaction (PCR) assay to detect various *Babesia rossi* genotypes. FrepBrEMA1 (5'-CCA ACA TTG ATG ATG ACA A-3') and RrepBrEMA1 (5'-CTG CAT GTC AGC TTA ATC A-3') were used to amplify a 460- to 520-base pair fragment of the 18S SSU rRNA spanning the V4 region. PCR/RLB assay results revealed that 96 (96%) of the 101 amplified parasite DNA hybridized to *Babesia rossi* species-specific probe and three (3%) to *Babesia vogeli* species-specific probe. Mixed infection was detected whereby two (2%) specimens were co-infected with *B. vogeli* and *B. major*. Based on sequence analysis, we have identified 9 BrEMA1 genotypes in agreement with previously published results and the existence of an additional 3 new genotypes. No amplification was observed in DNA from *B. vogeli* and *B. canis* samples. Although we were successful in detecting BrEMA1 genotypes using SYBR Green, the assay failed to reliably differentiate amongst the various genotypes. Correlation between clinical signs and BrEMA1 genotypes was studied. The most encountered clinical sign is collapse followed by hypoglycaemia, icterus and in-saline agglutination. Our results have shown that genotype29 is associated with seven (70%) out of 10 collapse cases and is associated with 62.5% of the dogs that died. The infection was higher in dogs of two years old or younger than dogs more than 3 years of age.

Identification of conserved genes between *Rhipicephalus (Boophilus) microplus* and *R. (B.) decoloratus* tick species using DNA microarray

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Ticks are obligate haematophagous ectoparasites, affecting livestock around the world in tropical and subtropical regions. *R. (B.) microplus* (Canestrini, 1887) and *R. (B.) decoloratus* (Koch 1844) are two prominent species found to co-infest livestock in South Africa. Currently, chemical acaricides remain the predominant form of tick control, despite the growing prevalence of acaricide resistance and ongoing environmental contamination. To date, only a single vaccine has been commercialized against *Boophilus microplus*. This vaccine, based on an 86kDa GPI-anchored midgut protein, has limited efficacy against other related tick species and this needs to be addressed, since co-infestation is usually the situation in the field. In this study, a custom oligonucleotide microarray spanning all known nucleotide *B. microplus* sequences was designed. RNA from the midgut of *B. microplus* and *B. decoloratus* was converted to cDNA and labelled with Cy3 and Cy5, respectively. Data analysis revealed some 3,000 transcripts with no significant change in expression levels among the two species. These transcripts are representative of possible cross-protective vaccine candidates that need to be further investigated. This methodology can also be applied to search for genes that are conserved among other livestock-specific ticks with vaccine design in mind.

Identification and small-scale animal vaccination trials of promising protective antigens against the cattle tick *Rhipicephalus (Boophilus) microplus*

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Rhipicephalus (Boophilus) microplus is regarded as the most important tick species affecting the livestock industry and its economical sustainability. This species is known to transmit both the pathogenic *Babesia bovis* and less pathogenic *B. bigemina* species, as well as *Anaplasma marginale* and *Borrelia theileri*. To date, only a single vaccine candidate (Bm86) is available for vaccination against this tick species with a second candidate (subolesin) in trials. Our research focus on using functional genomics approaches, such as the yeast two-hybrid and in vivo RNAi, to identify new vaccine candidates. Firstly, by means of the two-hybrid system we identified three interacting partners of subolesin. These were further evaluated by in vivo RNAi and only one interacting partner, Elongation factor 1, was shown to have a significant phenotype. Secondly, in vivo RNAi analysis of six TFPI-like Kunitz proteins identified a single candidate (BmTFPI-1) with a significant phenotype. Subsequent expression of recombinant Elongation factor 1 and BmTFPI-1 proteins were performed and validated in small-scale cattle vaccination trials. Results identified BmTFPI-1 to decrease both the number and weight of engorged females, by 74% and 50% respectively. BmTFPI-1 is currently being further evaluated as a promising protective vaccine candidate against *R. (B.) microplus*.

Comparative phylogeography of the four-striped mouse, *Rhabdomys pumilio* and its specific parasitic louse, *Polyplax arvicanthis*

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Parasite-host interactions may lead to varying levels of congruence between parasite and host geographic genetic structures, ranging from complete incongruence to instances of strict co-speciation. The level of congruence is usually a reflection of the intimacy of the interaction between the host and parasite species, as determined by life history, ecological and demographic traits. In the current study the phylogeographic structures of the Four-striped mouse *Rhabdomys pumilio*, (Rodentia: Muridae) and its parasitic sucking louse *Polyplax arvicanthis* (Phthiraptera: Anoplura) were determined and compared for potential congruence. *Polyplax arvicanthis* possess several traits (such as host specificity, no free living phase or intermediate host as well as high levels of prevalence and abundance) that suggest congruent structure with the host. Host and parasite material was sampled from 13 localities across the distribution of *R. pumilio* within South Africa and Namibia. Sequencing of the mitochondrial gene COI and the nuclear gene EF1 α 1 was performed for a total of 307 host individuals. For the parasite, 315 individuals were sequenced for the COI gene and subsequently sequencing of 98 individuals, representing the mtDNA haplotypes, was performed for the nuclear gene CAD. *Rhabdomys pumilio* was found to consist of three well supported clades/species that are geographically structured according to vegetation Biomes. The Orange River and Western Great Escarpment seem to act as contemporary gene flow barriers. Within *P. arvicanthis*, the existence of two highly divergent clades is supported by both the mtDNA and nuclear data. The levels of divergence are comparable to that between other, recognized *Polyplax* species which may indicate that *P. arvicanthis* consists of two morphologically cryptic species. Significant geographic differentiation also exists within each of these divergent louse clades. The parasite and host geographic structures are broadly congruent, as predicted by their close biological association.

Mitochondrial DNA markers: a genetic approach to host-parasite interactions

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The relationships between parasites and hosts have often been studied due to the potential impact these may have on the health of domestic animals, wild-life and humans. The generalist parasitic mite, *Laelaps giganteus* (Mesostigmata) is a widespread southern African parasite, which generally occurs on a wide variety of small mammal species in this region. Morphological descriptive characters could however not determine if multiple lineages are present within *L. giganteus*. Several studies in southern Africa have suggested that geographically wide spread species are often cryptic species that have developed over time possibly as a result of habitat fragmentation and isolation. This study is aimed at recording the phylogeographic structure of the mesostigmatid mite, *L. giganteus*, on the rodents *Rhabdomys pumilio* and *R. dilectus* in South Africa. Here we analysed 30 individuals from two locations (Lainsburg and Oudtshoorn) in South Africa to present the first genetic data set describing the phylogeographic structure of *L. giganteus* using the mitochondrial COI gene. Upon analysis of the data high haplotype diversity was retrieved as well as elevated nucleotide diversity. No genetic differentiation was evident between the two populations, suggesting stable populations are maintained in *L. giganteus* with high effective population sizes. Currently *L. giganteus* therefore exhibits a population genetic structure which is expected from a generalist parasite.

Expression profiling gene silencing and transcriptional networking of Metzincin metalloproteases in the cattle tick *Rhipicephalus (Boophilus) microplus*

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Tick proteins functioning in vital physiological processes such as blood meal uptake, digestion and reproduction are potential targets for anti-tick vaccines, since vaccination could inhibit these functions and ultimately affect tick survival. In this study we evaluated metzincin metalloproteases from *R. microplus* as vaccine candidates since they are implicated as essential during blood-cavity formation, bloodmeal digestion and reproduction. Eight transcripts encoding proteins that contain the characteristic metzincin zinc-binding motif and the unique “methionine-turn” were identified from an in-house assembled *R. microplus* EST database. These were representative of five reprotolysin-like and three astacin-like metzincins. RT-PCR indicated that the reprotolysins were most abundantly expressed in the salivary glands, whereas the astacins were most abundant in the midgut and ovaries. In vivo gene silencing was performed to assess a possible phenotype of these metalloproteases during adult female *R. microplus* blood feeding and reproduction. RNAi against two of the reprotolysins and one of the astacins significantly affected the average egg weight and oviposition rate. Evidently, this reverse genetic approach enabled the evaluation of the overall vital impact of tick proteins. Finally, integrated real time-PCR studies also revealed an extensive cross organ network between the *R. microplus* metzincin transcripts, supporting the use of these metzincins in a possible combinatorial vaccine.

Characterisation of selected *Culicoides* (Diptera: Ceratopogonidae) populations in South Africa using genetic markers

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Culicoides (Diptera: Ceratopogonidae) are small (<3mm) blood feeding flies. These flies are biological vectors of viruses, protozoa and filarial nematodes affecting birds, humans, and other animals. Among the viruses transmitted those causing bluetongue (BT), African horse sickness (AHS) and epizootic haemorrhagic disease (EHD) are of major veterinary significance. *Culicoides* (*Avaritia*) *imicola*, a proven vector of both AHS and BT, is the most abundant and wide spread livestock-associated *Culicoides* species in South Africa. Field isolations of virus and oral susceptibility studies, however, indicated that a second *Avaritia* species, *C. bolitinos* may be a potential vector of both BT virus and AHS virus. Differences in the oral susceptibility, which are under genetic control, of populations from different geographical areas to viruses may be an indication of limited contact between these populations. A good knowledge of the distribution, spread and genetic structure of the insect vector is very important in understanding AHS or BT disease epidemiology. Therefore, it became important to identify a suitable genetic marker that can reveal possible subpopulations of *C. imicola* and *C. bolitinos*, and to identify genomic or mitochondrial DNA sequences that can be used in phylogeographic and population genetic studies. The results of the DNA analyses which included PCR, sequencing and phylogenetic analysis for the two species indicate that at least one subpopulation of *C. imicola* does exist in South Africa. This will, however, need to be confirmed by further studies.

Enhanced detection of African trypanosomes in blood and cerebrospinal fluid by LAMP

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The loop-mediated isothermal amplification (LAMP) assay with its advantages of cost effectiveness, rapidity and simplicity has evolved as a sensitive and specific method for the detection African trypanosomes. While sufficiently sensitive to detect trypanosomes DNA well below the amount present in a single parasite, the detection limit of the assay is physically dictated by the number of parasites present in the volume of sample assayed. It is hypothesized that clinical sensitivity that mimics analytical limits based on parasite DNA could be approached by simply adding detergent to the samples prior to LAMP assay. For proof of principle, LAMP assays capable of detecting 0.1 fg genomic DNA (0.01 parasite) were used. The assay was tested on dilution series of intact bloodstream form African trypanosomes in human blood or cerebrospinal fluid (CSF) or blood with or without the addition of the detergent Triton X-100. With human CSF and in the absence of detergent, the LAMP detection limit for live intact parasites using 1 µL of CSF as the source of template was at best 103 parasites/mL. With detergent, the LAMP assays could detect 1 to 10 parasites/mL or less. Similar detergent-mediated increases in LAMP assay sensitivity was found using DNA extracted from filter paper cards containing blood samples spotted on detergent pretreated cards. This simple procedure for the enhanced detection of live African trypanosomes in biological fluids by LAMP paves the way for the adaptation of LAMP for the economical and sensitive diagnosis of other protozoan parasites and microorganisms.

Detection of *Salmonella clostridium perfringens* and *Escherichia coli* from faecal samples of captive animals at the National Zoological Gardens of South Africa

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Escherichia coli, *Clostridium perfringens*, and *Salmonella* species are rod-shaped, opportunistic, enterotoxigenic bacteria with a ubiquitous distribution. Diseases caused by these pathogens are well recognized in humans, livestock, and zoo animals and can result in morbidity, mortality, and significant economic losses worldwide. The aim of this study was to identify and simultaneously amplify enterotoxigenic *E. coli* (ETEC), *Salmonella* species, and *C. perfringens* using multiplex PCR which amplifies the heat-labile (LT) and heat-stable (STa and STb) strains of *E. coli*, *Salmonella* spp. and enteric bacteria *C. perfringens* from fecal samples of captive animals. Bacterial DNA was isolated using the Zymo Research Fecal DNA kit from fecal samples of birds (n=17), primates (n=6) and antelopes (n=26) collected from the National Zoological Gardens of South Africa. From the bird samples 2/17 (12%); 1/17 (6%); and 1/17 (6%) were positive by PCR for the presence of *C. perfringens*; *Salmonella* spp.; and *E. coli* (STb) DNA respectively. Forty six (93%) of the 49 samples were all negative. The results were compared to post-mortem cases of NZG's potential zoonotic pathogens. The detection of these bacterial pathogens is an indication of the occurrence of the infections and calls for a detailed epidemiological study over an extended period as the prevalence of infection might be influenced by season or a variety of other factors.

Transcriptome analysis of immature life-stages and adult tissues of the cattle tick *Rhipicephalus (Boophilus) microplus*

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Rhipicephalus (Boophilus) microplus ticks are regarded globally as economically important due to their increasing resistance to acaricides, adaptability to different climatic zones and repertoire of pathogens transmitted. An effective strategy to manage acaricide-resistance and disease transmission is currently lacking. The development of new anti-tick vaccines requires immediate attention in order to complement current control methods and lessen the impact of this species. In an effort to expand the current repertoire of candidates, transcriptome analysis using DNA microarrays was performed as part of a systematic approach to identify transcripts expressed during feeding in more than one tissue and life stage of the life cycle of the cattle tick, *R. (B.) microplus*. A custom oligonucleotide microarray chip was designed representing 13,446 assembled *B. microplus* contiguous sequences that were derived from all available nucleotide sequence databases. DNA microarray analysis was performed using a reference design with tissues obtained from whole engorged larvae and nymphs, as well as female adult tick tissues (including salivary glands, gut and ovary tissues). We successfully identified 6,200 transcripts that are expressed in larvae, nymphs and adult tissues. By incorporating additional immuno-informatical analysis, promising new anti-*B. microplus* vaccine candidates have been identified. These will be further validated using small-scale animal trials.

Survey of equine piroplasmosis in Nigeria using molecular assays

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A total of 152 blood specimens were collected from apparently healthy horses and donkeys from North eastern Nigeria. Specimens were obtained from 98 horses and 54 donkeys. All the specimens were initially screened for *Theileria equi* and *Babesia caballi* using the *T. equi*-specific and the *B. caballi* – specific qPCR assay. The amplification of a region of the 18S rRNA gene was used for the detection of *T. equi* and *B. caballi*. Subsequently, these same specimens were further screened for the presence of *Babesia/Theileria* and *Ehrlichia/Anaplasma* genomic DNA using PCR and reverse line blot assays. The results of the survey shows that using qPCR, 92(93.88%) of the horses and 52(96.30%) of the donkeys were positive for *T. equi*. Conversely, 1(1.02 %) of the horses and 4(7.41%) of the donkeys were positive for *B. caballi*. The RLB results showed 89(90.82%) of the horses to be positive for at least one or more parasites while 50 (92.59%) of the donkeys were positive for at least one or more parasites. These study reports the first molecular survey of equine piroplasmosis in Nigeria. The results also show that the equine population harbour varied haemoparasites, some of which are reported for the first time in Nigeria.

Sequence variation and molecular phylogeny of novel *T. buffeli*-like and *T. sinensis*-like genotypes of the African buffalo (*Syncerus caffer*) based on their 18S rRNA gene and Internal Transcribed Spacer (ITS) sequences

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In a previous study, we identified *Theileria buffeli* from samples originating from the African buffalo (*Syncerus caffer*) in South Africa. The aims of this study were to characterize South African *T. buffeli* 18S rRNA gene and complete ITS (ITS1-5.8S-ITS2) sequences, determine the level of genetic variation between any novel *T. buffeli*-like genotypes and known *T. buffeli*-like genotypes, and to establish their phylogenetic positions based on the 18S rRNA gene and ITS sequences. Ribosomal DNA was amplified from DNA extracted from blood samples originating from buffalo in the Hluhluwe-iMfolozi Game Park (HIP) and the Addo Elephant Game Park (AEGP), and cloned, and recombinants were sequenced. We identified novel *T. buffeli*-like 18S rRNA gene and ITS genotypes from buffalo in the AEGP, and novel *T. sinensis*-like 18S rRNA genotypes from buffalo in the HIP. Phylogenetic analyses indicated that the *T. buffeli*-like sequences were similar to *T. buffeli*-like sequences from cattle and buffalo in China and India, and the *T. sinensis*-like sequences were similar to *T. sinensis* 18S rRNA sequences of cattle and yak in China. There was extensive sequence variation between the novel *T. buffeli* genotypes of the African buffalo and other *T. buffeli*-like and *T. sinensis*-like genotypes. This is the first report on the characterization of the full-length 18S rRNA gene and ITS region of *T. buffeli*-like and *T. sinensis*-like genotypes in South Africa. The presence of organisms with *T. buffeli*-like and *T. sinensis*-like genotypes in the African buffalo is of significant importance, particularly to the cattle industry in South Africa, as these animals might act as sources of infections to naïve cattle. Our study provides important information which will assist in the classification of the complex *T. buffeli*/*T. sergenti*/*T. orientalis* group of benign and mildly pathogenic species.

Poster presentations:

Serpins and the mosquito's immune response to pathogen challenge

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The female *Anopheles* mosquito is exposed to diverse blood-borne pathogens during a blood meal. In most instances it initiates molecular and cellular immune responses against these pathogens to overcome invasion. For example, when infected with *Plasmodium*, immune responses to lyse or melanise the parasites are initiated. Fungal infection results in the formation of antifungal peptides as well as melanisation of the invading fungi. Various immune genes are involved in this process of which serine proteases are examples. They are involved in initiating and propagating mosquito defence responses some of which, if left unregulated, can often lead to mosquito death. These proteases activate melanisation which results in the formation of products and by-products that are toxic not only to invading pathogens but to the insect host itself. It is in this role that serine protease inhibitors (serpins) exhibit their importance as they control serine protease activity, thus preventing deleterious effects from occurring to the mosquito host. This study aimed to identify serpin genes within *An. funestus* and the effects of pathogen challenge on their expression levels. Since only partial genome information of the subject is available, degenerate primers were developed to identify *An. funestus* specific serpin genes. Sequencing confirmed the identity of these genes and quantitative real time PCR was used to analyse the effect of pathogen challenge on their expression patterns. Understanding the molecular action of serpins in *An. funestus* will provide essential information that can be used in vector control intervention strategies.

Gene sequencing of a Kunitz-encoding vaccine candidate in the cattle tick *Rhipicephalus (Boophilus) microplus*

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Rhipicephalus (B.) microplus tick species are of global medical and veterinary importance due to the number of parasites transmitted by these species and their economic impact on livestock. Identification and understanding of promising vaccine candidates against this species is vital to control disease transmission and livestock losses. BIR-1 was identified as a promising vaccine candidate via gene silencing and vaccination trials. Silencing of BIR-1 resulted in the up regulation of two additional genes, BIR-2 and BIR-3. We hypothesize that these three genes share similar promoter areas, which are activated by identical transcription factors. This study utilized chromosome walking, genome complexity reduction and affinity methods in order to identify the gene sequence and upstream region of BIR-1. Chromosome walking identified the gene sequence of BIR-1. The 3'-intron region was subsequently used as a biotin labelled probe for affinity purification of genomic DNA sequences encoding BIR-1 coding- and flanking sequences. DNA sequencing of affinity purified genomic DNA segments identified a retro-transposon rich area upstream of BIR-1. Further studies are in progress to further reduce genome complexity and optimize affinity purification techniques in order to obtain putative upstream promoter sequences for the three BIR-genes.

Relative real-time PCR validation of putative reference genes and gpi-linked membrane protein transcription profiles from *Rhipicephalus (Boophilus) microplus* and *R. (B.) decoloratus*

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Current vaccination with the GPI-linked glycoprotein Bm86 lacks cross-protective properties and effectiveness in different geographical regions, despite Bm86 being a relatively conserved antigen. Further discovery of new vaccine targets is vital in order to reduce environmental pollution by acaricides, overcome acaricide resistance and to minimize livestock losses due to ticks and tick-borne diseases. Only two reference genes are currently validated for the use in *R. (B.) microplus* using qBASE software. The discovery of novel household genes in *Boophilus* species is thus important in order to obtain accurate and reliable expression profile data. DNA Microarray analyses of some 13,464 contigs during the various lifestages of *R. (B.) microplus* identified a significant amount of household genes and some 29 promising GPI-linked membrane protein vaccine candidates. Relative real-time PCR (q-PCR) validation of the observed expression levels was performed using cDNA from two immature *R. (B.) microplus* life stages, three tissues from mature *R. (B.) microplus* and gut tissue from *R. (B.) decoloratus* adult females. Results indicated microarray expression profiles from non-saturated spots to be identical to qPCR results. These households will be invaluable for future expression profiling studies. The GPI-transcript is currently being expressed for validation as vaccine candidate.

Development of a LAMP assay for the specific detection of *Theileria* sp. (sable) in sable (*Hippotragus niger*) and roan (*Hippotragus equinus*) antelope

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Alarming decreases in numbers of sable (*Hippotragus niger*) and roan (*Hippotragus equinus*) antelope populations have been shown in the past decades which can be attributed to a combination of disease, drought-caused food shortages, habitat loss and degradation, and subsistence hunting. Sable antelope occur in scattered populations in dry open woodlands and medium-tall grass savannas in East and Southern Africa. Some populations have been established outside the historic range of the species, while breeding populations are held in a number of zoos throughout the world. Roan antelope formerly occurred very widely in savanna woodlands and grasslands of sub-Saharan Africa, but has been eliminated from large parts of its former range. The species remain locally common in West and Central Africa, while in East and Southern Africa, the traditional antelope strongholds, the species is now very rare. At present about one-third of the species' total population occurs in protected areas. It is well known that wild ruminants harbour a wide variety of piroplasms, some of which are pathogenic, while others are considered moderately pathogenic or benign. Piroplasms are tick-borne intracellular apicomplexan parasites which inhabit erythrocytes, and sometimes other cells, of vertebrates. *Theileria* and *Babesia* are the two main genera and contain many species of major veterinary and economic importance. Mortality in juvenile sable and roan antelopes in South Africa has been reported to be associated with a *Theileria*-like species. Fatal cases of theileriosis occurring after translocation in sable and roan antelopes have also been reported. The cause of death in the sable and roan antelope was attributed to the presence of *Theileria* sp. (sable). Microscopic examination is still a common approach for the detection of theileriosis but with a low sensitivity. Although the Reverse Line Blot (RLB) assay has successfully been used to detect *Theileria* sp. (sable) in sable and roan antelope, it is laboratory-based assay and requires specific equipment to be performed. The Loop-mediated isothermal amplification (LAMP) assay is, however, more sensitive and results can be obtained in less than an hour. It can also easily be performed in a field laboratory. The aim of the study was to develop a sensitive LAMP assay for the specific detection of *Theileria* sp. (sable) in sable and roan antelope. Two primer sets were designed using the Primer Explorer V2 software package; one set for the detection of all *Theileria* species, which was successful, and the other for specific detection of *Theileria* sp. (sable). Preliminary results indicated that the latter assay was not specific, thus requiring further optimization and/or the selection of another target gene for the development of a *Theileria* sp. (sable) specific assay.

A *Babesia odocoilei*-like parasite in a Siberian tiger

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Babesia is an intracellular erythrocytic haemoprotozoan of mammals but has also been reported in reptiles and birds. The two most frequently reported *Babesia* species in felids are *B. felis*, which causes clinical babesiosis in domestic cats, *B. leo* and *B. lengau*, primarily reported from asymptomatic lions and cheetahs, respectively. In-house blood smears from “Tara”, a Siberian Tiger (Jukani, Wild Life Sanctuary, Western Cape Province) revealed the presence of parasites resembling *Babesia bovis* but certainly larger than most of the *Babesia* spp found in cats (including *B. felis*, *B. leo* and *B. lengau*) and smaller than the large *Babesia* group (*B. rossi*, *B. vogeli*, *B. bigemina* and *B. caballi*) and *B. presentii*, reported from cats in Israel. The animal was sick at time of blood collection but recovered after 2 days. EDTA blood was collected, and submitted for molecular analysis to the Molecular Laboratory, Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, Onderstepoort, South Africa. DNA was extracted; the V4 hypervariable region of the parasite 18S rRNA gene was amplified and subjected to the Reverse Line Blot (RLB) hybridization assay. The PCR products were subsequently cloned, sequenced and subjected to phylogenetic assays. The RLB results indicated the presence of a *Babesia* or *Theileria* parasite. Sequencing and phylogenetic analysis showed highest similarity (94%) with *B. odocoilei* previously isolated from farmed elk and reindeer, from Wisconsin, USA.

Hyperparasites or hypersymbionts?

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Phoresis, mutualism, commensalism and parasitism are the textbook terms used to describe the living condition of two organisms in close association. Hyperparasitism is another well-known phenomenon amongst parasitological associations. Although scientists have wordy definitions for all of these associations, the organisms living in these relationship most of the time does not fit one hundred percent into our textbook definitions. Algae are normally found free living, but numerous forms are found symbiotic in other invertebrates e.g. those found in giant clams, others are hypersymbionts, such as the zooxantellae that are found in *Mantoscyphidia* (Ciliophora) which occurs on the gills of limpets. The carapace of species of the genus *Doropygus* (Copepoda) that are endosymbionts of tunicates, are sometimes covered with sessiline ciliophorans, which have numerous zooxantellae inside them. The occurrence of hypersymbiotic associations between members of the same group (ex Protozoa) is well known. *Epistylis* (Ciliophora) have been found to attach to the scopula of *Apiosoma* (Ciliophora), which occur on the skin of fish. *Ellobiophrya* (Ciliophora) species have originally been described as symbionts of non-ciliophoran hosts, but a new species have recently been found to attach with the cinctum to the scopula of *Mantoscyphidia*. The hyperparasitic association of protozoans on or in invertebrates have also been well documented. *Plasmodium* (Apicomplexa) species, found as parasites in the gut and salivary glands of mosquitoes, where the latter feed on the blood of the vertebrate host hence also a parasite, is probably the most commonly known example of hyperparasitism. Parasitic crustaceans such as *Dolops*, *Opistolernaea* and *Lernaea*, which occur on the skin or, in the gill chambers of fishes, have been found to be hosts for *Epistylis* species. One of the more interesting examples of hyperparasitism is found amongst the genus *Udonella* (Monogenea), which was described from the carapace of caligids that are parasitic on the skin of marine fish. Until the late nineties the taxonomic position of *Udonella* was more in dispute than the fact if this monogenean was a hyperparasite or just living in a symbiotic association on the carapace of *Caligus*. Hyperparasitism was thought to be one of the options of biological control of *Gyrodactylus salaris* (Monogenea) found on Atlantic salmon. An unidentified rod-shape bacteria and *Ichthyobodo necator* (Kinetoplastida) were found on *G. salaris* collected from salmon. The intensity of *I. necator* (an ectoparasite found more attached to the salmon than to *G. salaris*) was however never high enough on the monogeneans and turned out to be an unlikely candidate for biocontrol of gyrodactylosis. To date most of the hyperparasites recorded from helminths are representatives of the Protozoa. The associations described above and the many others that exists, should be seen in the context of their trophic status within the ecosystems were they occur, rather than to classify them into ill-fitting man made categories.

Detection of helminth infections from canine faecal samples in Qwaqwa, South Africa

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The aim of the study was to determine prevalence of canine helminth infections in selected areas of Qwaqwa's Maluti a Phofung Municipality. Faecal samples collected from domestic dogs (n=100) in Qwaqwa villages were analysed for the presence of helminth infections using the faecalizer and McMaster techniques. Sampled villages were Mphatlalatsane (n = 52) and Qholaqhwe (n = 38) as well as a semi urban area, Clubview (n = 10). A total of 37% of the samples tested positive for the presence of different helminth egg species namely, *Toxocara canis* (7/100), *Ancylostoma caninum* (4/100), *Taenia* spp (2/100), *Uncinaria stenocephala* (16/100), *Toxascaris leonina* (6/100) and *Trichuris vulpis* (2/100). The EPGs ranged from 133 – 4666, 200 – 5800, 0 – 2666, 66 – 1733, 133 – 3933 and 466 – 1066 for *T. canis*, *A. caninum*, *Taenia* spp, *U. stenocephala*, *Toxascaris leonina* and *T. vulpis* respectively. Of the 37/100 positive faecal samples 10/37 (27%) were successfully cultured to 1st stage larvae. This study has documented important information about the presence of canine helminth parasites in Qwaqwa and brings about opportunities for large scale epidemiological studies in the area.

Identification and molecular characterization of *Ehrlichia* and *Anaplasma* species of the African buffalo (*Syncerus caffer*)

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Anaplasmosis and heartwater are the most important diseases of ruminants worldwide causing significant economic losses in the livestock industries in tropical and subtropical areas. The African buffalo is known to be the reservoir host of numerous important tick-borne pathogens, of which some are carried asymptotically and can cause disease to susceptible animals. *Theileria parva*, (causes East Coast fever, Corridor disease and January disease), *Ehrlichia ruminantium*, (heartwater), and *Anaplasma marginale* (which causes anaplasmosis), are considered to be the most important tick-borne disease agents of livestock worldwide. Little is known about the *Ehrlichia* and *Anaplasma* spp. infection status of African buffalo; hence the primary objective of this study was to determine the occurrence of these species in buffalo blood samples collected from two game parks in South Africa (Kruger National and Hluhluwe-iMfolozi Park). The samples were simultaneously screened for the presence of *Theileria*, *Ehrlichia*, *Anaplasma* and *Babesia* spp. DNA was extracted from 200 buffalo blood samples originating from the Kruger National and Hluhluwe-iMfolozi Parks in South Africa, and subjected to the reverse line blot (RLB) hybridization assay. Results obtained, revealed the presence of *Anaplasma centrale*, *A. marginale*, *Ehrlichia* sp. *Omatjenne*, *Babesia occultans*, *T. parva*, *Theileria mutans*, *Theileria* sp. (buffalo) and *Theileria velifera*, either as single or as mixed infections. A major finding is the presence of *A. centrale* and *A. marginale* suggesting that buffalo are a natural reservoir of *Anaplasma* spp. infection and could play an important role in the epidemiology and spread of anaplasmosis and may represent a serious threat to the livestock industry. Since none of the samples tested positive for *E. ruminantium* with the RLB hybridisation assay they were subjected to a quantitative real-time PCR (qPCR) assay using the pCS20 gene region for the specific detection of *E. ruminantium*. Only 2 % tested positive, suggesting maybe that buffalo are not the natural reservoir host of *E. ruminantium* and that other wildlife spp. need to be investigated as possible reservoir hosts. Furthermore, in a number of samples, the PCR products did not hybridize with any of the *Anaplasma/Ehrlichia* and/or the *Babesia/Theileria* species-specific probes used in the RLB, but only with the genus-specific probe, suggesting the presence of novel species or variants of a species. These are currently under investigation.

The detection of *Anaplasma marginale* infections in cattle in the Gauteng Province, South Africa

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Bovine anaplasmosis is a tick-borne disease caused by intraerythrocytic rickettsial pathogen *Anaplasma marginale*. It is the most prevalent tick-borne infection of cattle with a worldwide distribution particularly in tropical and subtropical areas. It is endemic in South Africa and the disease results in considerable economic loss in susceptible cattle. Samples were collected in 11 communal farms in the Gauteng province. This study was designed to determine the prevalence of bovine anaplasmosis in different communal farms of the province. Microscopic examination of blood smear, serology and molecular diagnosis techniques were used for the detection of this pathogen. Two pair's of primers targeting the msp1; and msp4 genes were used for the amplification of the pathogen. Out of 109 blood samples tested, 92 (84%) were positive by microscopic examination, only 3 (3%) were positive with PCR when using msp 4 gene. Forty one samples were chosen randomly for serological analysis with cELISA and 73% were seropositive. These results indicate high prevalence of anaplasmosis in the Gauteng province. It is therefore evident that cattle in this province conform to infection rates that are indicative of a situation approaching stability.

Monogenea infesting the gills of *Labeo lunatus* from the Okavango River Botswana

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Monogeneans are ectoparasites of both marine and freshwater fish where they are found on the gills and skin of their hosts. Monogenean parasites are strictly host and site specialists that may occur naturally in high infestations. These parasites are equipped with a unique attachment organ (opisthaptor) at the posterior end of the body and are said to be not very harmful to their hosts, but usually cause epizootics under culture conditions. In Africa, the genera *Dactylogyrus* and *Dogielius* are represented by monogeneans only infesting the family Cyprinidae. The objectives of this research were to identify and study the morphology of *Dactylogyrus* and *Dogielius* spp. infesting the gills of *Labeo lunatus* from the Okavango River, Botswana. Field collections of the host were made at different localities in the River. *Labeo lunatus* collected with the aid of hand nets were anaesthetised using clove oil, measured, dissected and the gills removed for monogenetic parasite examinations. Monogeneans were collected, mounted in ammonium picrate whereafter the opisthaptor and reproductive organs were studied by light microscopy. Two monogenetic parasites of *Dactylogyrus* Diesing, 1850 and one *Dogielius* Bychowsky, 1936 species were found to infest the gills of *L. lunatus* namely: *Dactylogyrus* spp. 1 and 2 and *Dogielius* sp. 1. *Dactylogyrus* spp. 1 and 2 shows distinct variations in the opisthaptor, especially the shape of the first hooklet. The most characteristic feature is the shape of the copulatory organ. The dorsal bar of the *Dogielius* sp. 1 is characterised by rounded ends whereas the dorsal bar of already described species are pointed. All three monogenetic species are the first records on *L. lunatus* for the Okavango River, Botswana and may be described as new species.