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The following are abstracts of papers and posters at the 42nd Annual Congress of the Parasitological Society of Southern Africa (PARSA) 22–24 September 2013, Stonehenge in Africa, Parys, Free State, South Africa.

Invited Lecture:

Polystomatidae studies in Africa: Past - Present – Future

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The monogenean family Polystomatidae Carus, 1863, represented by 23 genera, has radiated into a variety of aquatic and semi-aquatic tetrapod hosts. Of these 10 genera are known from the Ethiopian realm. They are *Eupolystoma, Kankana, Madapolystoma, Metapolystoma, Polystoma, Protopolystoma*, known from anuran hosts, *Neopolystoma, Polystomoidella* and *Polystomoides* from chelonians and *Oculotrema* from a mammal, the hippopotamus. The first polystome to be described from Africa was *Polystoma africanum* that was described from Liberia in 1932. In the 1960's a few French researchers started working in the francophone countries and described several species. Leading figures were Bourgat, Combes, Euzet, Knoepffler, Maeder, Murith and Salami-Cadoux. The first polystome from South Africa was described by Combes & Channing in 1978. In 1984 Dawie Kok from the Free State University established a focus on this group of parasites. He remained active in the field for 10 years when Louis du Preez and co-workers took over. Studies in Madagascar revealed a unique diversity and two new genera *Madapolystoma* and *Kankana* was described from the island and more than 10 species awaits description. Recent molecular studies revealed a close ancestral link between *Nanopolystoma* from South American caecileans and *Oculotrema, Polystomoides* and *Neopolystoma* from Africa. A new challenge is to find a caecilian polystome in Africa.

Oral presentations:

Dactylogyrids (Platyhelminthes: Monogenea) from exotic Cyprinidae in Limpopo province, South Africa

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Monogenean flatworms are common parasites of aquatic cold-blooded vertebrates such as freshwater fishes and are mostly host specific with a cosmopolitan distribution. The suborder Dactylogyrinea is the most numerous and diverse group among the Monogenoidea. Some of these dactylogyrids are very harmful and responsible for high mortalities in fish, especially in culture conditions.

Exotic Cyprinidae from Limpopo province, South Africa, were checked for monogeneans. Dactylogyrids were found on common carp (*Cyprinus carpio* Linnaeus, 1758), goldfish (*Carassius auratus* Linnaeus, 1758) and koi (*Cyprinus carpio haematopterus,* Linnaeus, 1758). An interesting point is that most goldfishes and kois that are sold in Limpopo province are coming from other provinces (especially Gauteng) and in some cases even from overseas, like Israel. Uncontrolled import of live fishes (like ornamental aquarium fishes) into the country can lead to transmission of pathogenic monogeneans or other groups of parasites to native fishes that can be a serious economic and ecological threat to valuable native fishes.

Several species of monogeneans have been introduced and imported into Africa with their specific hosts, such as *Dactylogyrus anchoratus* and *Dactylogyrus extensus* with common carp. Prevalence and mean intensity in examined fishes were as follows: Carp 90.0% and 12.9, Goldfish 85.7% and 6.7 and Koi 60% and 5.2. Occurrence of monogeneans on exotic cyprinid hosts and the risk of introducing exotic pathogens with importing fishes or any other living organism to the country are discussed in this study.

Monogeneans and marine aquaculture in South Africa

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Finfish production still contributes very little to the overall marine aquaculture production in South Africa. However, there is considerable investment toward the development of this sector into a viable industry. Currently dusky kob (*Argyrosomus japonicus*) has reached commercial scale production with further interest being expressed toward further developing Silver Kob (*Argyrosomus inodorus*), Yellowtail kingfish (*Seriola lalandi*), white stumpnose (*Rhabdosargus globiceps*), yellowbelly rock cod (*Epinephelus marginatus*), spotted grunter (*Pomadasys commersoni*) and mangrove snapper (*Lutjanus argentimaculatus*). The basic identifications and taxonomic data for the parasites or parasitic taxa that cause disease in aquaculture systems form the foundation for disease diagnoses and applied epidemiology. In addition to this, these data are fundamental for effective disease management; the assessment of disease risks and impacts posed by the movement of aquatic animals; and the establishment of aquaculture facilities and aquaculture development zones. The relevant biological and ecological requirements, of parasites posing the greatest risk to the culture of the respective host species, both naturally and within different culture systems and conditions are also required in order to develop and assess pathogen specific control measures and optimise the efficacy thereof . This overview provides a summary of recent knowledge and discoveries on monogeneans encountered on prospective aquaculture candidate finfish species in South Africa and include elements of epidemiology, taxonomy and systematics, biology, integrated management strategies and treatment trials for monogeneans from both wild and captive reared fish.

Parasite host switching from invasive American turtles to native turtles in their natural environments

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Throughout the world freshwater turtles are traded for the food market and as pets. It's estimated that between 1988 and 1994 roughly 26 million turtles were exported worldwide. Though some countries banned turtle imports, 3-4 million turtle hatchlings are still annually exported from the USA. Some turtles are released or escape into the environment. Upon release of these turtles, feral populations could establish. As with all introductions they pose a threat to local biodiversity and ecosystem functioning. In France, *Trachemys scripta elegans* is considered as an invasive threat to the Mediteranean pond turtle (*Mauremys leprosa*) and the European pond turtle (*Emys orbicularis*) as they may compete for resources and habitat.

Freshwater turtles are host to a variety of parasites including haemoparasites, nematodes, flukes and cestodes. When invasive turtles escape, parasites may spread to native species. The objective of this study was to document the extent of polystomatid flatworm invasions from *T. s. elegans* to natural turtle populations in southern France and northern Spain. Also to examine the risks that host switching might pose on indigenous species. Our studies revealed that *T. s. elegans* serves as a taxi for a variety of polystomes from other American turtles. We also found evidence that *M. leprosa* throughout the south of France are infected by several non-native polystomes. It was thus evident that host switching did take place in natural environments. It would appear that turtle polystomes are not as strictly host-specific as initially thought. The global trade in freshwater turtles thus provide opportunity for parasites to be transported to new destinations and exotic parasites could impact the physiology, behaviour and survival of native turtle species.

Ectoparasitic *Nerocila* Leach 1818 (Isopoda, Cymothoidae, Crustacea) species of South African fishes

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Members of the genus *Nerocila* Leach, 1818, are fish-parasitic isopods easily observed on external surfaces of their fish hosts. Identification of species in this genus can be problematic as the large amount of variation observed within *Nerocila* is not fully understood, leading to many species being incorrectly synonymised or misidentified due to these variable characteristics. Currently, only five species of *Nerocila* are known from South Africa. After deep sea and estuary studies along the southern and eastern coasts of South Africa, *Nerocila* specimens were collected and identified. Two known species were found to be new for the South African fauna and found on new hosts. The first species, *N. monodi*, was collected in the St Lucia estuary on *Argyrosomus hololepidotus* (Madagascar meagre), while the second species, *N. depressa*, was collected from the Thukela estuary on the external surfaces of *Thryssa vitrirostris* (Orangemouth anchovy) and *Hilsa kelee* (Kelee shad). Another three species, *N. orbignyi, N. phaiopleura* and *N. sigani*, were confirmed for the region and new hosts for these isopods were identified. *Nerocila orbigyni* was found on *Chelidonichthys capensis* (Cape gurnard); *N. phaiopleura* was removed from the external surfaces of *Thryssa vitrirostris* (Orangemouth anchovy); while *N. sigani* was found on *Argyrosomus japonicus* (Mulloway)*, Thryssa setirostris* (Longjaw thyrssa)*, T. vitrirostris* (Orangemouth anchovy) and *Johnius dorsalis* (Small kob). Furthermore, after studying all of the *Nerocila* material preserved in the Iziko South African Museum, Cape Town, it can be concluded that *N. serra,* which was thought to occur in South Africa, was incorrectly identified and is not in fact located in this region.

Morphology and phylogeny of clinostomatid parasites from *Tilapia sparrmanii* and *Oreochromis mossambicus*

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Metacercariae of *Clinostomum* and *Euclinostomum* species belonging to the Family Clinostomatidae are often referred to as yellow grubs, due to the colour of their gut contents. They are usually found encysted in the muscle and buccal cavity of many species of freshwater fish worldwide. Metacercariae of *Clinostomum* species have been the subject of several taxonomic revisions due to the high degree of morphological variability within the same species. The use of molecular methodologies has allowed links of various developmental stages of these parasites to be elucidated. This project was aimed at studying clinostomatid metacercariae collected from freshwater fishes using morphology and PCR analysis. Collected specimens of clinostomatid metacercariae from freshwater fish were fixed in 70% ethanol, stained in haematoxylin/eosin and Van Cleave's haematoxylin. Micrographs were taken using a Nikon Coolpix®990 digital camera. Specimens for PCR were suspended in 5% saline buffered solution and frozen in a – 70 ^{0}C freezer until required. Extracted DNA samples were amplified, sequenced and analysed. Clinostomatid metacercariae morphologically identified as *Clinostomum tilapiae* were collected from the buccal cavity of *T*. *sparrmanii.* Phylogenetically the length of the sequenced region was 462. Readable regions of rDNA partial sequences of this parasite were as follows: 5.8S region had nucleotides starting from 1-111 (partial); ITS-2 gene started from 112-397 and it covered the whole ITS-2 region (complete) and 28S gene started from 398- 462 (partial). Euclinostomatid metacercariae were found embedded in the muscle tissue of *O*. *mossambicus* and *T. sparrmanii*. This was a preliminary study to evaluate the possibility of applying molecular biology techniques to the investigation of larval digenetic parasites. In addition to the morphological descriptions, the application of molecular techniques on digenetic trematodes seems very promising and may yield great potential in future descriptions of these parasitic species.

Alien parasite invasion in the Eastern Cape: Threat to economically important freshwater fishes?

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The impacts of alien fish species on indigenous fish species include the transfer of associated parasites, direct predation on indigenous fish and ecosystem effects resulting from changing invertebrate community structure. As a result alien invasive fishes are considered the primary threat to the native fish fauna in South Africa. However, a paucity of information exists on the effect of alien parasites on economic fish species. Therefore the aim of the study was to determine the effects of alien parasites on economic important fish species from various impoundments in the Amatola region of the Eastern Cape, South Africa. Three dams, Binfield Park Dam, a 260ha dam, Sandile Dam, 146ha dam and Wriggleswade Dam, a 1000ha impoundment were sampled in July 2011, March 2012 and August 2012. The fish were collected using gill and fykes nets. Live fish were returned to the field laboratory, externally examined for any macroscopic parasites, where after sacrificed and dissected. The dissected fish were then examined for any internal macroscopic parasites. All parasites found were preserved as prescribed for that specific group. The Fish Health Assessment Index (FHAI) was applied to determine the general health status of all the fishes. Fish collected and screened for parasites were *Labeo umbratus*, *Labeobarbus aeneus* and *Anguilla mossambica.*

The alien monogenean, *Pseudodactylogyrus anguillae* was found on the gills of *Anguilla mossambica* from all three the impoundments. The anchor worm, *Lernaea cyprinacea* was found infesting only *Labeo umbratus* from Sandile Dam and both *Labeobarbus aeneus* and *Cyprinus carpio* from Wriggleswade Dam. The tapeworm, *Bothriocephalus acheilognathi* was found infecting *Labeobarbus aeneus* from Wriggleswade Dam however, it was not found infecting *Cyprinus carpio* from Wriggleswade Dam. Although *Cyprinus carpio* are not present in Binfield Park or Sandile Dam, however, its associated parasite *L cyprinacea* was present on the fish in Sandile Dam. Despite the presence of the above mentioned alien parasites results from the FHAI showed that all the different species studied were in a healthy state. However, it is important to note that of all the fishes sampled during this study only *Anguilla mossambica* is indigenous to the Eastern Cape. Therefore further research into more of the indigenous fish species inhabiting the Eastern Cape Rivers is required to determine the full threat of these alien parasites.

Epidemiology of the most probable fish helminth in Limpopo province, South Africa

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Contracaecum (Anisakidae), based on long term studies done on different fish species in the Limpopo province, is the most prevalent helminth in fishes across the province. This typical anisakid larva is found in the body cavity and mesenteries of fish and the adult is present in the alimentary canal of fish-eating birds. The aim of the present study was to conduct a preliminary epidemiological survey of fishes for infections with *Contracaecum* larvae in Limpopo reservoirs.

During 2006 to 2013, a total of 15 species of fishes from nine families from different localities across the Limpopo province were examined for nematode larvae. These include *Micropterus salmoides* (Centrarchidae), *Hydrocynus vittatus* (Characidae), three species of Cichlidae (*Oreochromis mossambicus*, *Tilapia rendalli*, *Tilapia sparrmani*), *Clarias gariepinus* (Clariidae), five species from the Cyprinidae (*Barbus mattozi*, *Barbus trimaculates*, *Cyprinus carpio*, *Labeo rosae*, *Labeobarbus marequensis*), *Glossogobius giuris* (Gobiidae), *Synodontis zambezensis* (Mochokidae), *Marcusenius macrolepidotus* (Mormyridae) and *Schilbe intermedius* (Schilbeidae) from nine different localities (reservoirs) across the province (Molepo, a tailings dam at Anglo Platinum, Phalaborwa Barrage, Flag Boshielo, Nandoni, Nwanedi-Luphephe, Tompi Seleka, Matlala and Tzaneen). The highest prevalence (78%) and mean intensity (750) of *Contracaecum* was seen in *Clarias gariepinus*.

Contracaecum larvae were found in the abdominal cavity of all examined fishes, except *T. sparrmani*, *B. trimaculates*, *L. rosae*, *G. giuris* and *S. zambezensis*. Ongoing research will include studies on the life cycle of this nematode; most importantly the definitive hosts (fish eating birds) from different localities. Thus far *Contracaecum* adult worms were found in *Phalacrocorax lucidus* (White-breasted cormorant) and *Anhinga rufa* (African darter) while no worms were present in a single *Butorides striatus* (Green-backed heron) examined at Flag Boshielo Dam.

The functional micromorphology of the flea *Demeillonia granti* from the eastern rock elephant shrew *Elephantulus myurus*

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Elephant shrew species are the primary hosts of *Demeillonia granti* which has been collected throughout the arid western half of southern Africa, as well as from the Namaqua rock mouse sharing their rocky habitat. This investigation using the scanning electron microscope (SEM) sought to confirm the taxonomic characteristics of *D. granti* as well as illucidating the functional micromorphology. The fleas were collected from *Elephantulus myurus* trapped on the Ezemvelo Nature Reserve, Mpumalanga and fixed in 70% ethanol. Some of the fleas were cleared and mounted for identification under the LM, while the rest were routinely processed for SEM before being viewed in a Zeiss Supra 55 FE-SEM.

D. granti are elongated fleas with the males measuring under 1.66 mm in total length and the females approximately 2.2 mm. The frons of the head was angular bearing fine setae anterior to the five prominent flattened spines forming the genal comb. The shape of each was characteristic, with the third spine greatly elongated and tapering to a point. The spines were angled for hooking onto the hairs of the host. The large eyes were situated anterio-dorsally and protected by the frons. The antennae were large and held in the deep antennal grooves by spatulate suckers in the male. The mouth parts composed of two serrated laciniae and a rodlike epipharynx for penetrating the skin, were housed in a pair of tube-like labial palps. The prominent pronotal comb consisted of stout round apically flattened bristles (12 in the male and up to 18 in the female) longitudinally ridged for hooking onto the hairs. The tibia of the hind legs bore stout spines arranged in 9 dorsal notches reminiscent of a comb, which is a characteristic of this species. The tarsal claws with lateral plates were specialized with ventral grooves for gripping the hairs of the host. The spiracular openings on the abdominal tergites were elongated except for the large slit-like posterior spiracles on tergite VIII. The sensilium and complex male and female external genitalia were also investigated. A number of micromorphological specializations of *D. granti* were illucidated in this SEM study.

Taxonomic reassessment of terrestrial tortoise haemogregarine, *Haemogregarina fitzsimonsi* Dias, 1953, with identification of its vectors

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Two haemogregarine genera are known to infect chelonians, *Haemogregarina* (*sensu stricto*) and *Hemolivia*. *Haemogregarina* (*s. s.*) species, according to Siddall's (1995) taxonomic review, are solely transmitted by leech vectors. However, this appears improbable for terrestrial South African tortoises, from which leeches are absent. Ticks, however, such as *Hyalomma aegyptiam*, known to transmit the apicomplexan *Hemolivia mauritanicum* between Palearctic tortoise species are more plausible vectors. It was therefore, hypothesized that *Haemogregarina fitzsimonsi* Dias, 1953, a common South African tortoise haemogregarine is transmitted by tick vectors and furthermore, may need to be taxonomically reassessed. Blood collected from 270 tortoises of 5 genera and 7 species was used to prepare thin, fixed and stained smears. Films were screened for parasites, identified as *H. fitzsimonsi*. Ticks from tortoises were fixed in 70% molecular grade ethanol, and possible *H. fitzsimonsi* sporocyst and sporozoite stages were identified on dissection of adult *Ambylomma sylvaticum* and *A. marmoreum* ticks from apparently infected and non-infected tortoises. Parasite DNA was extracted from both slides and ticks using a DNeasy Animal Tissue Kit and PCR was undertaken with three primer sets: HEMO1 and HEMO2, HepF300 and HepR900 and 4558F and 2773R. Results indicated that apicomplexan DNA extracted from blood films and both species of tick had been amplified by one or more primer sets. *Haemogregarina fitzsimonsi* 18s rRNA sequences aligned with those of species of *Hepatozoon* and this is the first report of an *Hepatozoon* from any terrestrial chelonian. Furthermore, *H. fitzsimonsi* appeared to be neither intermediate host nor vector specific, perhaps having the ability to infect novel tortoise and tick hosts. Since tortoises are popular in the pet trade, and are not necessarily kept free of ectoparasites in these circumstances, there may be reason for concern for naive tortoise species' welfare.

Preliminary results on the biodiversity of South African amphibian blood parasites

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Studies have shown that frogs host a diversity of parasites. At present hardly any information exists on blood parasites or haematozoans parasitising South African frogs. We decided to undertake a study aiming at: 1) documenting blood parasite diversity, 2) provide host and locality records of described and new haematozoan species, and 3) describe new parasites using morphometrics and DNA analysis. Frogs were collected through active sampling at night. Blood was obtained by means of puncture of the femoral artery. Thin blood smears were prepared, air-dried, fixed in methanol, and stained using Giemsa's stain. Micrographs of parasites were captured using a Nikon eclipse E800, high resolution compound microscope and measurements taken at 100x magnification. To date 25 frog species have been examined from five localities including the Drakensberg Mountains, Ndumo Game Reserve, Phongolo floodplain, Potchefstroom and Cape Town, across three different provinces namely KwaZulu-Natal, North-West and the Western Cape. Fourteen species were found to be infected with haematozoans: 11/14 (79%) with haemogregarines, 6/14 (43%) with trypanosomes, 1/14 (7%) with babesiasomes, and 1/14 (7%) with microfilaria. It is hoped that these and future results may increase the knowledge of apicomplexans parasitising amphibian hosts in South Africa.

Molecular diagnosis and phylogenetic analysis of *Babesia bigemina* and *Babesia bovis* haemoparasites from cattle in South Africa

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Babesia parasites, mainly *Babesia bovis* and *B. bigemina*, are tick-borne haemoparasites inducing bovine babesiosis in cattle globally. The clinical signs of the disease include, among others, anemia, fever and haemoglobinuria. Babesiosis is known to occur in tropical and subtropical regions of the world. In this study, we aim to provide information about the occurrence and phylogenetic relationship of *B. bigemina* and *B. bovis* species in cattle from different locations in nine provinces of South Africa. A total of 430 blood samples were randomly collected from apparently healthy cattle in different locations throughout South Africa. These samples were genetically tested for *Babesia* parasitic infections using nested PCR assays with species-specific primers. Neighbour-joining trees were constructed to study the phylogenetic relationship between *B. bigemina* and *B. bovis* sequences of randomly selected isolates. Nested PCR assays with Group I primer sets revealed that the overall prevalence of *B. bigemina* and *B. bovis* in all bovine samples tested was 64.7% and 35.1%, respectively. Only 117/430 (27.2%) animals had a mixed infection. The highest prevalence of 87.5% for *B. bigemina* was recorded in the Free State province collection sites (Ficksburg, Philippolis and Botshabelo), while North West collection sites had the highest number of animals infected with *B. bovis* (65.5%). Phylograms were inferred based on *B. bigemina*-specific *gp45* and *B. bovis*-specific *rap-1* nucleotide sequences obtained with Group II nested PCR primers. Phylogenetic analysis of *gp45* sequences revealed significant differences in the genotypes of *B. bigemina* isolates investigated, including those of strains published in GenBank. On the other hand, a phylogeny based on *B. bovis rap-1* sequences indicated a similar trend of clustering among the sequences of *B. bovis* isolates investigated in this study. This study demonstrates the occurrence of *Babesia* parasites in cattle from different provinces of South Africa. It was also noted that the situation of *Babesia* parasitic infection in cattle from certain areas within the surveyed provinces had either reached endemic stability or was progressing towards stability.

In silico predicted *Babesia bovis* antigens are indicative of potential candidate diagnostic antigens

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Babesia bovis is an obligate intracellular protozoan parasite, imposing important constraints on livestock health and economic development in tropical and subtropical regions throughout the world. This organism is responsible for the most prevalent and costly tick borne disease, namely bovine babesiosis. The current control approaches have many limitations enabling babesiosis to remain prevalent worldwide. In this study, we identified two novel genes of *B. bovis* encoding two proteins from *B. bovis*, named B.bov 16 and B.bov 22. The proteins are potentially novel and secreted proteins that may be used as diagnostic antigens. Bioinformatics analysis indicated that they have signal sequences, are hydrophobic, have multiple epitopes and are immunogenic suggesting that they may also be used as candidate vaccine antigens. Both genes were obtained by Polymerase Chain Reaction and ligated into the pJET 2.1 cloning vector. The recombinant plasmid constructs were identified by colony PCR and sequencing. The positive clones were double digested and inserted into the expression vector pGEX3x and recombinant constructs were expressed as GST–fusion proteins in the *E. coli* BL21 (DE3) cells. Analysis with SDS-PAGE revealed the correct sizes of the proteins as 42KDa and 48kDa for B.bov 16 and B.bov 22 respectively. Further studies will be undertaken to develop an ELISA diagnostic assay that will use these recombinant proteins as antigens and evaluate it with field samples from infected cattle.

Babesia lengau associated with cerebral and haemolytic babesiosis in two domestic cats

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Although reported sporadically from various countries, feline babesiosis appears to be a significant clinical entity only in South Africa, where *Babesia felis* is usually incriminated as the causative agent. *Babesia lengau*, recently described from asymptomatic cheetahs, has now possibly been incriminated as the causative agent in two severe clinical cases in domestic cats. Both cats were euthanised in extremis. While typical feline babesiosis in South Africa is an afebrile disease with a chronic manifestation, there was acute onset of severe clinical signs in both cats and their body temperatures were above the normal range when they were presented for treatment. Haemolytic anaemia was confirmed in one case. To our knowledge, this is the first report of cerebral babesiosis in cats. On reverse line blot 18S rDNA PCR products obtained from both cats showed positive hybridization profiles with the *B. lengau* species-specific probe. The two partial parasite 18S rRNA gene sequences obtained, showed high sequence similarity (99.9 %) to *B. lengau*. In a representative tree constructed by the neighbour-joining method using the two-parameter model of Kimura the two obtained partial 18S rDNA sequences and that of *B. lengau* formed a monophyletic group with *B. conradae* and sequences previously isolated from humans and wildlife in the western USA. All clinical cases of feline babesiosis in South Africa are not necessarily caused by *B. felis*. Other piroplasms, e.g. *B. lengau*, may be incriminated in clinical cases, especially those occurring outside the known endemic area.

Emergence of *Culicoides* spp at Onderstepoort Veterinary Institute (ARC-OVI)

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The importance of *Culicoides* species were once more highlighted with the emergence of Schmallenberg virus in Europe in 2011. It is known that *Culicoides* species are vectors of at least 60 different viruses affecting man and livestock. African horse sickness (AHS) occurs annually in South Africa during the summer months, with the highest incidence of cases being reported, during autumn (March to April). Traditionally it is said that disease outbreaks stops during winter after the first severe frosts. But according to the AHS trust cases of AHS were reported as late as June during the 2010/11 AHS season. From collections made across South Africa it is known that in certain areas *Culicoides* species can be collected throughout winter in low numbers. It is reported that the extrinsic incubation period for most viruses transmitted by *Culicoides* species is below 12°C. Researchers worldwide have shown that depending on the species activity is greatly reduced below 10°C and that the larval stages go into diapause during winter. An experiment was set up at the ARC-OVI to establish if *Culicoides* species are breeding during the winter months and when the numbers emerging start to increase. Emergence traps were set up and monitored regularly from June to December 2012. From June to the end of August 1 409 specimens of nine different *Culicoides* species emerged and up to December a further 3 757 emerged. The ratio of males to females was nearly 1:1 (48.7% males vs 51.3% females). It can be concluded that *Culicoides* species are breeding and emerging throughout winter at the ARC-OVI.

Molecular identification of amphistomes in cattle in South Africa and Zimbabwe using internal transcribed spacer two (ITS2) region of rRNA gene

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Amphistomes are digenetic trematodes (flukes) that infect ruminants and other groups of animals. Species of this group of parasites cause a disease known as amphistomosis. The taxonomic status of this group of parasites in Southern Africa is unknown and heavy infection by juveniles flukes have been shown to cause severe diarrhea in cattle. The objective of this study was to identify amphistomes collected from cattle in South Africa and Zimbabwe using molecular method and compare with the morphological descriptions of the already documented species. Type-specific PCR and sequencing of the internal transcribed spacer of rRNA gene was used and nucleotide sequencing of ITS2 region of rRNA gene revealed the presence of four species of amphistome species; namely *Calicophoron daubneyi*, *Calicophoron microbothrium Gastrothylax sp*. and one unidentified *Calicophoron spp*. We intend to morphologically confirm if this unidentified group is indeed a new species. The analysis also found that majority of animals sampled showed mixed infections while others had single infection.

Seroprevalence of bovine cysticercosis in cattle belonging to small-holder farms in Gauteng Province

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Bovine cysticercosis is a muscular infection of cattle caused by the larval stage of a zoonotic cestode, Taenia saginata. Currently, diagnosis of bovine cysticercosis is mainly by meat inspection; a postmortem examination which although useful in detecting cysticercosis in heavily infected carcasses, lightly infected carcasses may easily be missed and passed on for human consumption. Consequently, use of meat inspection records tends to underestimate the disease prevalence. Furthermore, being a postmortem examination, meat inspection does not avert financial loss and not all cattle slaughtered for human consumption are taken to abattoirs and inspected. It was therefore the aim of the current study to determine prevalence and distribution of bovine cysticercosis in Gauteng Province using an antemortem technique. Blood samples were collected for a period of twelve months from randomly selected cattle belonging to small-holder farm. A total of 1150 blood samples were collected from cattle with 543, 353 and 254 blood samples respectively collected from Pretoria, Germiston and Randfontein regional centres. Sera were aspirated from blood samples and analysed through a monoclonal antibody based HP10 antigen detecting ELISA (Harrison et al., 1989). This technique is three times more sensitive than meat inspection and detects parasite products associated with current infection. Results showed a high prevalence (26%) of Taenia infections from cattle which was distributed throughout the province. Pretoria had the highest prevalence (36%) when compared to Germiston (23%) and Randfontein (17%). Risk factors associated with Taenia infections in cattle include access to human excreta, which may be promoted by free roaming behaviour of cattle and absence of toilets. The presence and high prevalence of Taenia infections in Gauteng Province suggests that not everyone in the province utilises the available toilets. Furthermore, cattle are not always confined in kraals, but are let to roam freely during the day for grazing; this creates an infection opportunity through ingestion of Taenia contaminated herbage or water. Livestock management practices and limited use of toilets in the province therefore seem to be the major risk factors of bovine cysticercosis in the province. Public awareness programmes on life cycle T. saginata and use of more sensitive diagnostic tools to determine endemicity of the disease are also recommended as part of effective control strategies against Taenia infections.

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A SEM study of the micromorphology of the louse *Damilinia natalensis* Emerson 1963 from the nyala *Tragelaphus angasi*

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Lice collected from nyala in the Ndumu Game Reserve in Kwazulu-Natal, included chewing lice which had not previously been reported on nyala. These were identified as *Damilinia natalensis* Emerson 1963, described from the related bushbuck *Tragelaphus scriptus.* As this species is difficult to distinguish from *Damalinia hopkinsi* Bedford 1936 from the eland *Taurotragus oryx*, it was decided to do a scanning electron microscope (SEM) study to investigate the micromorphological characteristics of *Damilinia natalensis.* The lice were preserved in 70% ethanol. Some were prepared and mounted for identification by LM. The remainder were ultrasonically cleaned and routinely prepared for SEM and viewed in a Zeiss SUPRA55VP FE-SEM at 2 kV.

The head was shovel-shaped with a deep anterior V-shaped notch dorsally, formed by the deep ventral clypeal groove containing a pair of robust asymmetric mandibles. The three segmented antennae showed sexual dimorphism with the proximal segment being robust in the male, and the medial surface of the distal segment having two thickened conical setae and a serrated margin for attachment during copulation. The distal segment bore 11 sensory sensilla, two pit organs with internal tufts and three associated plate organs.

The thorax bore a pair of lateral mesothoracic spiracles and large ventral notal pits. The three pairs of legs each ended in a long slender tarsal claw which closed against a single thickened seta on the distotibial process. Seven broad abdominal tergites were identified each with a single posterior row of short setae. The posterior margins of tergites I and II were raised as irregular ridge-like processes, characteristic of male *D*. *natalensis* and *D*. *hopkinsi*. The bulbous paratergites II to VII bore the abdominal spiracles. The female gonopods VIII were uniquely sickle-shaped curving anterio-medially, and lined with a fringe of setae posteriorly. This SEM study revealed that the male gonopods VIII and IX formed a complex structure, with gonopods extending posteriorly as pairs of specialized lobes. These observations may be of morpho-taxonomic importance in distinguishing these related species.

Effects of host sex and pregnancy on *Trichinella zimbabwensis* infection in Sprage Dawley rats and Balb C mice

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Trichinellosis is an important zoonotic parasitic disease caused by nematode parasites of the genus *Trichinella*. The parasites infect a wide range of hosts including both domestic and wild animals. The main mode of transmission to human is through ingestion of undercooked pork meat. The successful establishment and development of the parasite in a host has been reported to be affected by environmental, immunological, physical and hormonal. The objective of this study was to determine the effect of host sex and pregnancy on the establishment and development of *Trichinella zimbabwensis* in Sprague Dawley rats and Balb C mice.

Fifty (50) Sprague-Dawley (25 males and 25 females) rats were orally infected with the parasite at a standardized dose of 1000 larvae/animal. At 5 day intervals from day of infection, five (5) animals from each group were sacrificed and the number of adult parasites in the intestine as well as larvae in muscles was counted and recorded. To determine the effect of host pregnancy on the successful establishment and development of the parasite, 60 Balb/C mice were orally infected with the parasite at a standardized dose of 50 larvae/gram of animal live weight. At seven (7) day intervals from day of infection, six (6) animals from each group were sacrificed and the number of adult parasites in the intestines as well as larvae in the muscles was counted and recorded. In addition, levels of progesterone and cortisol were measured at the same intervals.

The results obtained on the effect of host sex on establishment and development of the parasite showed that the parasite rate of establishment and development were significantly higher ($P < 0.05$) in males than in females. Determination of effect of host pregnancy on establishment and development of the parasite showed that pregnancy reduced the establishment and development of the parasite.

The results obtained are discussed in relation to the effect of host sex and pregnancy associated hormones on the establishment and development of *T. zimbabwensis*.

Assessment of selected biochemical parameters and humoral immune response of Nile crocodiles (*Crocodylus niloticus*) experimentally infected with *Trichinella zimbabwensis*

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Fifteen crocodiles were randomly divided into three cohorts of five animals each to represent high, medium and low infection cohorts represented by 642, 414 and 134 larvae/kg bodyweight respectively. The parameters assessed were blood glucose, creatine phosphokinase (CPK), lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT). The humoral immune response to *T. zimbabwensis* infection was evaluated in all three cohorts by an indirect ELISA method. The results showed deviations from normal parameters of blood glucose, CPK, LDH, AST and ALT when compared to reported levels in uninfected reptiles. Contrary to studies involving mammals, hypoglycaemia was not observed in the infected groups in this study. Peak values of blood glucose, LDH and AST were observed on day 56, 49 and 42 p.i. in the high, medium and low infection cohorts respectively. Peak values of CPK were observed on day 35 p.i. in all three cohorts. Peak ALT values were reached on day 56 in the high infection cohort and on day 28 p.i. in both the medium and low infection cohorts. No correlations between the biochemical parameters and infection intensity were observed. Peak antibody titres were reached on day 49 p.i. in the medium infection cohort and on day 42 p.i. in both the high and low infection cohorts. Infection intensity could not be correlated with the magnitude of the humoral immune response or time to seroconversion. Results from this study were in agreement with results reported in mammals infected with other *Trichinella* species and showed that antibody titres could not be detected indefinitely.

The efficacy of maslinic acid on *Trichinella zimbabwensis*-infected Sprague-Dawley rats

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Natural products have played a significant role as leads for the development of new drugs against intestinal parasitic worms. Recent studies have reported that maslinic acid (MA), a natural triterpene obtained from olive pomace, which has multiple biological, antimalarial, and antimicrobial activities, also exerts inhibitory effects on the development of some Apicomplexans which include *Eimeria spp*, *Toxoplasma gondii* and *Neospora caninum*. To date no studies have been carried out to determine if MA shows anthelmintic activity and hence the main objective of this study was to assess the efficacy of MA on *Trichinella zimbabwensis* Sprague-Dawley rats. Twenty four male Sprague-Dawley weighing 250 grams each were randomly selected and divided into four groups and all groups were infected with crocodile (*Crocodylus niloticus*)-derived *Trichinella zimbabwensis.* Group one was treated with MA (2.5mg/kg) on day 25 post infection, group two was treated with fenbendazole (FBZ) (7.5mg/kg) on day 25 post infection, group three was treated with a combination of MA and FE and group four acted as the control with no treatment. Blood was collected on a weekly basis for enzyme to determine the levels of LDH, CPK and also glucose from day 21 post infection. All rats were sacrificed on day 42 post infection. *Trichinella zimbabwensis* larvae in muscle were determine through pepsin digestion and the efficacy of MA was determined by comparing the number of muscle larvae among the experimental groups.

Results showed a significant reduction in larval counts ($P < 0.05$) in the treatment groups for both FBZ and MA when compared to the control group. After single treatment FE showed the most reduction in infection as compared to MA. LDH and CPK were elevated in the muscles in all groups after day 21 post infection. In conclusion MA showed some anthelmintic activity against *T. zimbabwensis* and further studies are recommended using higher doses.

Host behaviour and environmental factors shape nematode diversity in two co-occurring rodent species

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Small mammals are highly adaptable to environmental challenges and therefore form part of nearly all terrestrial communities and contribute significantly to biodiversity, food webs and maintaining ecosystems. Despite their ecological and economic importance little is known of the ecology that drives their helminth communities. In South Africa current information pertains mainly to taxonomic records. We investigated the prevalence and abundance of helminths of small mammals at two localities in Gauteng Province during summer 2012: Kaalplaas Nature Reserve and Rietvlei Nature Reserve, both within the Grassland/Savanna biome. Murids and soricids were collected in Sherman live traps with *Rhabdomys pumilio*, commonly called the four striped grass mouse and *Mastomys coucha*, the southern multimammate mouse, being the most common. Thirty *R. pumilio* and 18 *M. coucha* were trapped at Kaalplaas and 24 *R. pumilio* and 18 *M. coucha* at Rietvlei. Hosts were dissected and the gastro-intestinal tracts collected for helminth recovery and stored in 70% alcohol. Worms were cleared in lactophenol for identification. The nematode fauna of *R. pumilio* and *M. coucha* at the two study sites was moderately species rich and a total of eight nematode species, representing four orders, was recovered from *R. pumilio* and *M. coucha,* namely, *Heligmonina boomkeri*, *Neoheligmonella capensis*, *Syphacia obvelata*, *Syphacia* sp., *Ascarops* sp., *Protospirura muricola*, *Protospirura numidica* and *Trichuris muris*. Five of the eight nematode species were shared between the hosts. The distribution of parasites was aggregated as is often observed in parasitic infections. The overall prevalence of nematodes in *R. pumilio* was similar at the two localities (Kaalplaas 73%; Rietvlei 83%), but the overall mean abundance at Rietvlei was double that at Kaalplaas, 30.2 and 15.1 respectively. The overall prevalence in *M. coucha* at Kaalplaas (90%) was distinctly higher than that at Rietvlei (56%). The same held true for mean abundance, 51.8 and 13.5 respectively. Despite high worm burdens in individual hosts these hosts did not appear adversely affected.

Show me your teeth and I'll tell you who you are – or – Two new species of *Cylicospirura* Vevers, 1922 (Nematoda: Spirocercidae) in southern Africa

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Cylicospirura Vevers, 1922 was established to accommodate *C. subaequalis* (Molin, 1861) described from *Puma concolor* (Linnaeus) in Brazil, characterized by amongst other criteria, the presence of six large teeth projecting anteriorly in the buccal capsule. Since then a number of species have been described from felid, canid and dasyurid carnivores worldwide. In Africa, *C. subaequalis* was recorded from lions as early as 1929 and subsequently from leopards and hyaenas in eastern, western and central Africa. However, these records were not accompanied by descriptions and a recent morphological study of specimens of *Cylicospirura* from a leopard and hyaena in southern Africa suggests that earlier records might require further study. Two new species of *Cylicospirura*, *C. crocutae* Junker & Mutafchiev, 2013 from stomach lesions in a spotted hyaena, *Crocuta crocuta* (Erxleben), in Zimbabwe and *C. pardalis* Junker & Mutafchiev, 2013 from oesophageal lesions in a leopard, *Panthera pardus* (Linnaeus), in the Republic of South Africa were described. The former is distinct by having four cephalic and four external labial papillae, disposed at the level of the amphids, the presence of small accessory teeth between the six large tricuspid teeth that arm the buccal capsule and the fifth and the sixth pairs of the caudal papillae being equidistant from the cloaca. *Cylicospirura pardalis* is characterized by having tricuspid teeth with large, claw-like cusps, four cephalic and six internal labial papillae. Together with *C. felineus* (Chandler, 1925) whose buccal capsule is equally armed with tricuspid teeth and which was recovered from a stomach granuloma of a cat in South Africa, three of the currently eleven species recognized in the genus have been recorded from southern Africa. Given the diversity of large and small carnivores in the Afrotropical realm, it stands to reason that the diversity of *Cylicospirura* is even larger than currently recorded for this region.

Morphology and phylogenetic studies of trematodes and their potential threat to humans living around waterbodies in Gauteng and North-West provinces

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Trematodes are known to be host-specific as they inhabit most vertebrates as adults and their primary hosts are molluscs. However, agricultural and social practices have led to humans being accidentally infected with non-human trematodes. The present research project provides morphological information through microscopy and phylogenetic studies using polymerase chain reaction techniques on a number of parasites that may infect humans in the future. Parasitic stages were collected from freshwater snails, other invertebrate and vertebrate hosts. They were identified morphologically employing microscopy and PCR techniques. The parasitic stages collected were identified as: 1) clinostomatid cercariae and metacercariae; 2) echinostomatid cercariae, metacercariae and adults; 3) avian schistosome cercariae of the genus *Trichobilharzia;* 4) adults of *Fasciola gigantica* and 5) strigeid cercariae. Some of these parasites had similar morphological characterizations to parasites that were found to infect human beings in other parts of the world. The PCR amplification yielded bands ranging in size from ±400 bp to ±1500 bp. The amplicons contained only partial regions. The taxonomic identification using morphological characteristics has been found to be extremely difficult. The construction of phylogenetic trees successfully placed some of the parasites in their respective families. Parasite identification in the future must include both morphology and molecular aspects in order to identify trematodes successfully. The occurrence of several lesser known trematodes may pose serious health threats to communities residing around these natural waterbodies. It is therefore imperative to educate these communities as to the potential dangers and to implement necessary control measures.

An *Emoleptalea* species - what a unique trematode?

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An *Emoleptalea* sp. was found in the intestine of *Schilbe intermedius* at Nwanedi-Luphepe Dam in the Limpopo Province, South Africa. This group of parasites belong to the Family Cephalogonimidae, representing a group of small, spinous digenean parasites in the gastro-intestinal tract of fishes, amphibians and reptiles. The most characteristic feature of this parasite is the position of the genital pore at the anterior extremity of the body. The aim was to study the morphology of these *Emoleptalea* specimens sampled from the intestine of the silver catfish. *Schilbe intermedius* was collected from the Nwanedi-Luphephe Dam using gill nets. Fish were euthanized with clove oil, dissected and the intestine examined using a stereomicroscope. All parasites were fixed and preserved in 70% ethanol. Specimens were stained in Van Cleave's haematoxylin and material for scanning electron microscopy was routinely prepared and viewed in a VP FE-SEM at 2 kV. Adult *Emoleptalea* specimens were sampled from the anterior part of the intestine. They are small parasites (653 x 364 µm) and the entire tegument is covered with spines. The oral and ventral suckers are situated in the anterior half of the body. The pharynx is muscular and extends into the intestinal caeca that ends at the level of the posterior testis. The cirrus sac occupies the space between the ovary and the pharynx and contains a bipartite internal seminal vesicle, pars prostatica and long ejaculatory duct. Both the male and female genital openings exist to the outside close to the anterior extremity of the body. The hindbody of mature specimens is occupied by the uterus, filled with many small eggs. The vitelline follicles cluster together on either side of the ventral sucker. This is the first record of an*Emoleptalea* sp. in South Africa. Since the prevalence of this parasite is rather high at Nwanedi-Luphepe Dam with a mean intensity of 17.6 and range of 2 - 152, it would be of value to also search for the first and second intermediate hosts in the life cycle of this parasite.

Identification of diplostomid metacercariae in the eyes of *Tilapia sparrmanii* and the brain cavity of *Clarias gariepinus*, using morphology and molecular techniques

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Diplostomid metacercariae inhabit the eye lens, the retina, vitreous humor and the central nervous system of freshwater fish. The metacercariae of the genera *Diplostomulum* and *Diplostomum* are often morphologically similar. The determination of metacercariae by morphological trials is often difficult and ambiguous. The use of molecular methods has allowed links to be elucidated using various developmental stages of these parasites. The objective of this study was to identify diplostomid metacercariae collected from *Tilapia sparrmanii* and *Clarias gariepinus* using the morphology and Polymerase Chain Reaction (PCR) techniques. Freshwater fish were sampled from different dams, using a hand scoop and casting nets. The fish were kept in aerated aquaria and fed fish flakes. The eyes and the brain were removed and examined through a compound microscope for morphological identification. All the metacercariae were preserved in 70% ethanol prior to DNA extractions using Qiagen kit. Standard procedures for amplification of rRNA region were followed. The DNA amplicons were sent to Inqaba Biotech laboratory for sequencing and phylogenetic trees generated using software programs. Two dissimilar diplostomid metacercariae were collected from the eyes of *T. sparrmanii* and the brain cavity of *C. gariepinus* respectively. The amplicons of these diplostomids had band sizes of 500 bp. The amplicons contained only partial regions (ITS-2). Comparing the two diplostomid metacercariae in the present study with the ones described from various parts of the world, it can be concluded that the brain diplostomid belongs to the genus *Diplostomulum*. The eye diplostomid can only be classified to the family level.

Paraclinostomum elongatus gen. nov., sp. nov., of the family Clinostomatidae Luhe, 1901 from the oral cavity of domestic cats (_Felis catus domesticus_) from Mpumalanga province, South Africa based on molecular evidence

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Digenean flukes collected from the oral cavity of domesticated cats from Mpumalanga Province of South Africa were processed for identification using both morphological and molecular methods to determine their taxonomical classification. These samples were initially presumed to be *Clinostomum falsatum* based on the predilection site in the oral cavity. Both morphological and molecular data indicated that the flukes were not from the genus *Clinostomum* and represented a previously unreported new genus, *Paraclinostomum* gen. nov., sister to the genus *Clinostomum*, within the family Clinostomidae and the new species, *Paraclinostomum elongatus* sp. nov. Morphologically, the experimental samples differed from *Clinostomum* spp. in body shape, testes size and the distance between suckers. Molecular phylogenetic analyses based on 736 bp of the nuclear ribosomal internal transcribed spacer region (ITS1-5.8S-ITS2) showed that the flukes formed a 100% supported clade reciprocally monophyletic with and sister to already identified *Clinostomum* spp. The two clades were separated by a genetic distance of 15.7 to 17.2 %, consistent with an intergeneric distance. The results indicated that the flukes collected from the oral cavity of domesticated cats were not *Clinostomum* spp. but a new genus and species sister to the *Clinostomum* spp. In conclusion, the samples represent a new genus and species of digenean flukes from a genus not previously reported; further studies are required to elucidate the life cycle of this fluke.

Biogeography and host-related factors trump parasite life-history: limited congruence among the genetic structures of specific ectoparasitic lice and their rodent hosts

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Parasites and hosts interact across both micro- and macroevolutionary scales where congruence among their phylogeographic and phylogenetic structures may be observed. Within southern Africa, the four-striped mouse genus, *Rhabdomys*, is parasitized by the ectoparasitic sucking louse, *Polyplax arvicanthis*. Molecular data recently suggested the presence of two cryptic species within *P. arvicanthis* that are sympatrically distributed across the distributions of four putative *Rhabdomys* species. We tested the hypotheses of phylogeographic congruence and co-phylogeny among the two parasite lineages and the four host taxa, utilizing mitochondrial and nuclear sequence data. Despite the documented host-specificity of *P. arvicanthis,* limited phylogeographic correspondence and non-significant co-phylogeny was observed. Instead, the parasite-host evolutionary history is characterized by limited co-divergence and several duplication, sorting, and host-switching events. Despite the elevated mutational rates found for *P. arvicanthis,* the spatial genetic structure was not more pronounced in the parasite lineages compared to the hosts. These findings may be partly attributed to larger effective population sizes of the parasite lineages, the vagility and social behaviour of *Rhabdomys*, and the lack of host-specificity observed in areas of host sympatry. Further, the patterns of genetic divergence within parasite and host lineages may also be largely attributed to historical biogeographic changes (expansion-contraction cycles). It is thus evident that the association between *P. arvicanthis* and *Rhabdomys* has been shaped by the synergistic effects of parasite traits, host-related factors, and biogeography over evolutionary time.

African trypanosome interactions with the blood-brain barrier: A gene transcriptional study

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Human African trypanosomiasis (HAT) is a vector-borne parasitic disease that has a major impact on human health and welfare in sub-Saharan countries. Based on animal models, it is currently thought that trypanosome manifestation in the brain occurs by initial infection of the choroid plexus and the circumventricular organs followed days to weeks later by dissemination to the brain parenchyma. *In vitro* studies have shown that *Trypanosoma brucei* bloodstream forms rapidly cross the brain microvascular endothelial cells (BMEC) that form the blood-brain barrier (BBB) (1-3). Intravital brain imaging in mice has shown that bloodstream forms of *T. b. brucei* and *T. b. rhodesiense* enter the brain parenchyma within hours and the association of extravascular trypanosomes with postcapillary venules, but not capillaries, suggest that early brain infection occurs at the level of the neuroimmunological BBB (4). To better understand BBB factors that allow trypanosome entry into the central nervous system (CNS), we initiated a gene transcriptional study comparing human BMEC infected with the parasites under static or flow conditions that favor or impede HBMEC crossing. Through this analysis, we identified 43 candidate genes whose expression levels differ by 3SD, spanning 25 pathways. Overall, our data support previous findings and accentuate the importance of understanding how flow hemodynamics orchestrate the pathophysiological events that are signature to CNS HAT.

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Molecular characterisation of *Cryptosporidium* from animal isolates in the Limpopo province

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Cryptosporidiosis has caused major public health concerns especially in developing countries. However, very few studies have identified the genotypes responsible for infection in the north-eastern parts of South Africa. In the present study, a total of 361 animal stool samples were collected from three different villages in the Limpopo province namely, Makuleke village, Nkomo village and Ngove Village. The samples were subjected to a primary screening of *Cryptosporidium* using the modified Ziehl-Neelson acid fast staining method. A genotype-specific nestedmultiplex PCR reaction was used to amplify the actin gene for further identification and distribution of *Cryptosporidium* genotypes. The overall prevalence of *C. parvum* was found to be 47.4%, 25.0% for *C. andersoni* and 3.9% for *C. bovis*. Moreover, significant prevalence rates of mixed infections were detected with *C. parvum* and *C. andersoni* having the highest mixed infection prevalence of 13.2% compared to 1.3% between *C. parvum* and *C. bovis*. The most interesting mixed infection also had a 1.3% but comprised all three genotypes, *C. parvum*, *C. andersoni* and *C. bovis*. *Cryptosporidium parvum* had the highest prevalence among cattle with a prevalence rate of 61.5% whereas *C. andersoni*recorded its highest prevalence among goats with 38.5% and *C. bovis* also had the prevalence among goats with 7.7%. Moreover, *C. parvum* and *C. bovis* were more pronounced in young animals than in adults with 60.0% and 10.0% respectively compared to 48.6% for *C. parvum* in adultsand 3.1% for *C. bovis* in adults. Though there was no much difference, *C. andersoni* was more prevalent in adult animals with 25.0% compared to 20.0% in young animals. Furthermore, *C. parvum* was more prevalent in Nkomo village with 77.8%, *C. andersoni* in Ngove village with 50.0% and *C. bovis* in Makuleke village with 5.0%. This geographic distribution dominance of the three genotypes may be key in providing effective diagnostic and control hints of cryptosporidiosis in the Limpopo province. The findings of this study imparts baseline information on the distribution of *Cryptosporidium* genotypes in the Limpopo province and so more molecular epidemiology studies need to be done to substantiate these findings.

Molecular detection of haemoparasites in dogs in Mnisi Mpumalanga province, South Africa

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Canine vector-borne diseases have a worldwide distribution and are increasingly significant as emerging diseases. They are caused by various pathogens and transmitted via the bites of arthropods like ticks and fleas. The aim of this study was to screen for haemoparasites in blood samples collected from domestic dogs in the Mnisi area of Bushbuckridge, Mpumalanga Province in South Africa. A total of 122 blood samples were collected from October 2011 through May 2012 and spotted onto FTA filter cards. All samples were screened for the presence of *Ehrlicia, Anaplasma*, *Theileria* and *Babesia* species using a reverse line blot (RLB) hybridization assay.

Results indicated that 21/122 (17.2%) of the samples investigated were positive for *Ehrlicia canis*, 12/122 (9.8%) for *Babesia rossi*, while 5/122 (4.09%) were positive for *Babesia vogeli*, 26/122 (21.3%) of samples were positive for the genus-specific probe 1 of *Babesia*, 18/122 (14.75%) were positive for the genus-specific probes of Theileria/Babesia, 3/122 (2.45%) were positive for the genus-specific probe of *Theileria*, 1/122 (0.81%) was positive for the genus-specific probe 2 of *Babesia*. The remaining majority of samples only reacted with the genus-specific probes for *Ehrlicia*/*Anaplasma* 64/122 (52.4%). Haemoparasite DNA could not be detected in 40/122 (32.7%) of samples. These preliminary data indicated that *E*. *canis* are the most common haemoparasites of dogs in Mnisi. DNA samples that only reacted with genus-specific probes will be sequenced to determine if these are novel or variance of existing parasite species.

Molecular prevalence and genetic diversity of *Anaplasma marginale* isolates in South Africa

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Bovine anaplasmosis, caused by tick-borne rickettsia *Anaplasma marginale*, is endemic in tropical and subtropical regions of the world and results in economic losses in the cattle industry. The major surface protein 1a is one of the six MSPs identified on *A. marginale* that has been widely used to characterize the genetic diversity of this pathogen worldwide and is encoded by a single copy gene. MSP1a contains a variable number of the tandem repeated peptides in the amino-terminal region, while the remainder of the protein is highly conserved among isolates. The number of repeats varies among geographic isolates of *A. marginale* but is constant within an isolate and has been used as a stable genetic marker of isolate identity. The analysis of MSP1a gene repeat sequences, which has provided evolutionary information about geographically distinct *A. marginale* strains was used in this study to characterize pathogen genetic diversity in South Africa using sensitive and specific MSP1a gene PCR method for *A. marginale* The sequence of 45 *A. marginale* MSP1a samples were analysed and differences were found in the tandem repeat sequences and on the structure of MSP1a among the different isolates included in this study. MSP1a tandem repeats analyses resulted in 25 new sequences with changes in amino acid and 43 different strains of *A. marginale* were found in all analysed sequences. The results of this study provide further evidence of the genetic variability of *A. marginale* in South Africa and this confirm the value of MSP1a gene as a tool for further epidemiological and virulence studies that might contribute to designing effective control strategies for bovine anaplasmosis in South Africa and worldwide.

Occurrence of tick-borne pathogens from ticks and blood of small ruminants in the Free State and Eastern Cape provinces, South Africa

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Ticks and tick-borne diseases are major constraints in the development of the South African agricultural industry. Livestock, which form about 50% of the agricultural output in the country are very important to the livelihoods of the people. This study aimed to determine the prevalence of *Anaplasma marginale, Babesia bovis*, *B. bigemina* and *Theileria* species in ticks and blood collected from small livestock through the use of PCR. While there have been numerous studies concerning these pathogens amongst cattle, not much has been done on small ruminants. Positive detection of *A. marginale* was observed in 7.4% of the Eastern Cape (EC) goat-tick samples and in 3% of the Free State (FS) sheep-tick samples; 6.5% and 17.5% *Babesia* / *Theileria* / *Hepatozoon* species were detected in FS and EC respectively; *Theileria* spp. were detected amongst 44% goat-ticks in the EC as well as in 4% and 31% of the goat and sheep ticks in the FS respectively. Three out of twenty two (14%) of the sheep blood tested positive for *Theileria* spp. Sequences of *Theileria* spp. positive samples from sheep also matched with *Theileria* sp. (buffalo) and *T. parva* sequences in the nucleotide database. Characterization of the *Theileria* spp. positive samples revealed *T*. sp. (buffalo)-like and *T. parva*-like profiles with real-time PCR. Pathogens which have been reported mainly amongst cattle have been detected amongst ticks of small ruminants and infection confirmed in sheep blood in the current study. Planned future studies include epidemiology of the pathogens found, their possible pathogenecity as well as characterization of *Theleria* spp. in both ticks and sheep.

Tick-borne haemoparasite prevalence and *Theileria parva* strain diversity in the African buffalo (*Syncerus caffer*) population in the Chobe National Park and the Okavango Delta, Botswana

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The most important tick-borne diseases of livestock in central and southern Africa are theileriosis, heartwater, babesiosis and anaplasmosis, resulting in extensive economic losses to farmers in endemic areas. The African buffalo (*Syncerus caffer*) plays an important role as reservoir for these diseases. The most pathogenic and economically significant *Theileria* species in sub-Saharan Africa is *Theileria parva*, causing East Coast fever, Corridor disease and January disease in cattle. There are no official reports on the presence of *T. parva* in Botswana and information on detrimentally significant tick-borne haemoparasites especially in Northern Botswana is scarce. The main aim of the study was to screen buffalo samples from the Chobe National Park (n=64) and the Okavango Delta (n=57), Botswana for the presence of *Theileria, Babesia, Ehrlichia* and *Anaplasma* species using the reverse line blot (RLB) hybridization assay and for *T. parva* specifically using real-time PCR assay. The RLB results revealed that *Theileria, Babesia, Anaplasma* and *Ehrlichia* species, either as single or mixed infections, were present in both buffalo populations. Of the *Theileria* spp. present, *T. parva* (60%) and *T. mutans* (36%) were the most prevalent. Real-time PCR results indicated that 80% of the samples were infected with *T. parva.* The variable region of the p67 gene was subsequently amplified and the gene profiles were analysed in an attempt to characterize the *T. parva* parasites. Three p67 variants were identified, including typical cattle- and buffalo-derived p67 genotypes as previously detected in East African cattle and buffalo, respectively; as well as one of the novel genotypes recently described in the South African buffalo population. This confirm the findings of Sibeko *et al.* (2010) who found that p67 profiles are more complex than originally thought and could not be used to distinguish between cattle- and buffalo-derived *T. parva* isolates. Sequence data will have to be generated in an attempt to better understand the significance of these p67 genotypes in the epidemiology of theileriosis in Botswana.

Characterisation of the genetic diversity of the southern cattle tick, *Rhipicephalus microplus*, populations from South Africa

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Rhipicephalus microplus in an invasive cattle tick first introduced to South Africa during the Rinderpest epidemic of the 1890s. Since the first recorded displacement of native *R. decoloratus* by *R. microplus* in 1962, *R. microplus* has increased in its geographical spread and host range. Control of *R. microplus* with acaricides is becoming increasingly ineffective due to the rapid accumulation of acaricide resistance in tick populations within 2 years. This is hypothesised to be due to the accelerated adaptation of *R. microplus* at a genetic level. Therefore this study aims to determine the level, pattern and processes governing the genetic diversity of *R. microplus* populations in South Africa. Tick samples have been collected across South Africa in collaboration with Zoetis (previously Pfizer: Animal Science SA). Genomic DNA was extracted from ticks and their identity confirmed with ITS2 PCR-RFLP. The genetic diversity of *R. microplus* has been evaluated using the D3 region, ITS2 sequence and the COI gene. The D3 region was conserved amongst all samples, including closely related species to *R. microplus* and thus had no resolution. The ITS2 sequence yielded 8 haplotypes in South Africa (11 including outgroups) and indicated no geographical or ecological structuring of *R. microplus* in South Africa. The ITS2 data indicated that the tick populations of South Africa are expanding and that the Eastern Cape populations contained the highest genetic diversity. Phylogenetic analyses of the COI gene resulted in two haplotypes for *R. microplus*; a single haplotype for South Africa, West Africa and Brazil and another for America (difference by 1 nucleotide), also indicating no geographical or ecological structuring of *R. microplus*. This may be due to the large importation of *R. microplus* during the 1890-1960s resulted in a genetically similar population throughout South Africa. Thus *R. microplus* tick populations display no population structure according to geography or ecology, indicating that other factors influence the adaptation of *R. microplus*. Therefore, additional studies will be conducted with the ANT gene, and microsatellite markers to determine whether tick populations structure according to acaricide resistance or vectoral competence of *Babesia bovis (*Asiatic redwater).

The effects of a flumethrin and fluazuron combination pour-on formulation used on cattle exposed to a boosted *Rhipicephalus decoloratus* and *R. microplus* population in the Eastern Cape region of South Africa

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The study was conducted to investigate the effect of introducing animals treated with a Flumethrin 1% and Fluazuron 2.5% (Drastic Deadline eXtreme® - Bayer) combination product on tick population on the pasture. Twenty-six cattle (n=26) were used in a study on a pasture with boosted tick numbers. The climate and tick population data for the pasture from the previous year was available. A group of 6 cattle was introduced onto the pasture and treated with Drastic Deadline eXtreme on Day 0, +63, +126 and +189. The animals were left to graze naturally on the pasture for the duration of the study (259 days). On 10 occasions (every 28 days), 2 untreated, tracer calves were introduced onto the pasture and left to graze naturally for 7 days, before being removed from pasture and tick burdens determined. Monthly tick drags on the pasture were also conducted and compared to the previous year, along with climatological data. The tracer animals showed a 75% (Day +63) to 100% (Day +146) reduction in the mean number of *R. decoloratus* ticks collected (95% CI 57.10 - 100), and a 97.5% (Day +35) to 100% (Day +146) reduction in *R. microplus* ticks collected (95% CI 98.78 - 100). When compared to the previous year in similar climatological conditions, the mean reduction in ticks numbers as determined by monthly drags ranged from 40.7% on Day +28 to 100% on Day +140 (95% CI 72.59 - 100). Tick numbers on the treated animals showed an initial sharp decline and were maintained at a consistently low level for the rest of the study. This study demonstrates that cattle treated with this acaricide combination are able to reduce the tick burdens on pasture.

A non-invasive DNA extraction method for parasitic nematodes in wild carnivores

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Spirocerca lupi is a parasitic nematode that causes spirocercosis in canids. Veterinarians in and around the Pretoria area are of the opinion that *S. lupi* is more prevalent in the areas surrounding the National Zoological Gardens (NZG) in Pretoria (Tshwane metropole). The aim of this study is to determine whether wild carnivores housed at the NZG serve as reservoirs of *S. lupi* thus affecting the prevalence of *S. lupi* in the adjacent areas. For this study, a method was designed to extract nematode DNA from eggs in faeces. Although egg shedding is sporadic and unpredictable, this approach is non-invasive and allows for easy sample collection without stressing the animals. The method allows for eggs to be separated from the faeces before homogenization and DNA extraction. Universal nematode primers for cox1 have been demonstrated to amplify *S. lupi*. These were used to determine whether nematode DNA was present. All four animals tested, namely striped hyena (*Hyaena hyaena*), lion (*Panthera leo*), cheetah (*Acinonyx jubatus*) and African wild dog (*Lycaon pictus*) showed positive amplification with these primers. These amplicons will be sequenced to identify what species of nematode was amplified from the extracted DNA. Whether *S. lupi* is present or not, the described method can be applied to other parasitic nematode studies where DNA must be obtained in a non-invasive manner.

Development of microsatellite markers for the parasitic nematode *Spirocerca lupi*

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The parasitic nematode, *Spirocerca lupi*, is known to cause spirocercosis in canids. It occurs mostly in regions with a warm, tropical climate and has emerged as a threat to the canine population worldwide. Early detection is not possible due to diagnostic limitations. Very few molecular studies have been conducted on *S.lupi* thus far and in order to gain additional perspective on disease spread, more fine-scale genetic analysis needs to be conducted. Microsatellites are ideal genetic markers for many areas of research since they are co-dominant, highly polymorphic, selectively neutral and show biparental inheritance. We developed a set of microsatellite markers for *S. lupi.* Microsatellite-enriched sequences were isolated from adult worm samples using the FIASCO (fast isolation by AFLPs of sequences containing repeats) protocol and Roche 454 sequencing. This method has been shown to be time and cost effective compared to cloning and Sanger sequencing, with thousands of sequences that contain repeats being obtained. Using only half of a pyrosequencing lane, 36 482 reads were obtained, of which 21 390 sequences (58.63%) contained repeats. After applying certain restrictions to obtain optimal markers, 233 primer pairs were designed for *S. lupi*. Of these, 20 loci were tested and ten polymorphic loci were identified. A single multiplex PCR reaction was designed to amplify all ten loci, making genotyping very efficient and cost-effective. Nine loci were deemed suitable for population genetic analyses, with an average of 9.89 alleles per locus (range: 6-18). Since the microsatellite markers designed in this study amplified *S. lupi* in domestic dogs (*Canis domesticus*), they were tested on worm samples obtained from jackals (*Canis mesomelas*). The results suggest that *S. lupi* is found in jackals as well. The markers were also successful in amplifying DNA from other closely-related species, namely *Cylicospirura felineus*, *Philonema onchorynchi* and *Gongylonema pulchrum*. These markers will be used further to assess population genetic variation of *Spirocerca lupi* in primary hosts from South Africa. In the long term, information on the ecology and evolutionary potential of *S. lupi* can help to gain insight on how to reduce the risk of infection and prevent the spread of resistant alleles.

Fish health and parasites from Molepo Dam, Limpopo Province: New geographical and host records

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Molepo Dam in the Limpopo Province is a small dam with several endemic and alien fish species. This dam is situated close to a large rural community and a substantial amount of subsistence fisheries take place at the dam. Ecological integrity of such a dam is thus important and the aim of the study is to examine the health and diversity of parasite of fish by using the Health Assessment Index (HAI) and condition factor protocols in relation to water quality. Five fish species, *Oreochromis mossambicus* (Peters, 1852), *Cyprinus carpio* (Linnaeus, 1758), *Tilapia sparrmani* (Smith, 1840), *Micropterus salmoides* (Lacépède, 1802) and *Clarias gariepinus* (Burchell, 1822) were examined monthly (February to August 2013). The weight and length were recorded and HAI values were assessed and calculated for each fish species. To verify the results of the HAI, water quality analysis is included and water samples were collected seasonally at four sampling sites of the dam. All metazoan parasites were collected, fixed and preserved using conventional methods. The following parasites were recorded from the different fish species: *Dactylogyrus* sp., *Cichlidogyrus* sp., *Acanthogyrus* sp., a gryporhynchid cestode larva, *Argulus japonicus*, *Dolops ranarum* and *Ergasilus* sp. The prevalence, mean intensity and mean abundance of parasite infection have been calculated for all fish species and no infection reached alarming high numbers, which is consistent with an unpolluted aquatic ecosystem. These results represent a first geographical record for this dam and the acanthocephalan a new and first host record for South Africa. Data on the overall health of this aquatic ecosystem will provide a basic foundation for any subsequent management, intervention, or monitoring thereof in the future.

Neopolysoma (Monogenea: Polystomatidae) egg production influenced by environmental temperature

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In freshwater turtles polystomes are found in the urinary bladder, cloaca, mouth, pharyngeal cavity and eye cavity. Parasite eggs are released in the water at a slow but continuous rate. Daily counts of eggs present in the water thus provide an indication of egg production. Egg production of *Neopolystoma* sp. and *Polystomoides* sp. in the urinary bladder and pharyngeal pouches respectively of *Mauremys leprosa* was monitored over a period of 26 days. Six *M. leprosa* were collected in the south of France near Perpignan and housed individually in plastic boxes containing water to the depth of about 20 mm. Containers were placed outside in an restricted area to expose them to environmental temperatures. A well-defined egg-laying rhythm was observed that correlated with fluctuation in temperature. Egg-laying continued both in warm and colder temperatures, with an increase as temperatures increased. We observed a + 48 hour delay in response to a change in temperature.

Fish ecto-parasites as bio-indicators of water quality in dams proximal to Medunsa Campus

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In recent years freshwater and marine ecosystems have suffered from pronounced pollution caused by direct discharge of effluent from industries, agriculture and urban sewage systems and this man-made problem is intensifying with time. Consequently, alternative measures of monitoring freshwater bodies are pursued. These include conventional methods of taking water quality assessment to monitoring water using bio indicators. This research project was aimed at studying the impact of water variables such as temperature and pH on the prevalence of parasitic infestations on *Tilapia sparrmanii* (banded tilapia) in the laboratory settings. A total of 115 *T. sparrmanii* were collected from the Medunsa pond, Boekenhoutskloof farm dam and Supersand dam. They were randomly placed in four different glass aquaria in the laboratory. The parameters such as water acidity, alkalinity and water temperature were monitored under laboratory settings for each tank. Fish were sacrificed and dissected on a regular basis and examined for any parasitic infection. The parasites obtained were air-dried on slides and stained in haematoxylin and silver-nitrate. They were identified using a Nikon compound microscope. Fish placed in acidic water were very sensitive to pH of 5.90 and a total of 15 perished after a day of being exposed to the acidic environment. Fish in the alkaline tank were mostly infected with monogenean parasites. At 25—35 ˚C temperature only *Trichodina* spp. was found, whereas in temperature ranging from 20—25˚C high infections of protozoans from the genera *Epistylis*, *Chilodonella* and *Trichodina* were found. Water parameters such as pH and temperature have an effect on the presence of parasites and thus can be used to monitor the water quality in freshwater systems.

Bioaccumulation of selected metals in *Contracaecum* and their host *Schilbe intermedius* from Flag Boshielo and Nwanedi-Luphephe dams

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Pollution, due to human activities such as mining, has adverse effects on both parasites and their host. The effects include poor water quality and as a result fish and parasites accumulate excess metals that are deposited from these anthropogenic activities. The aim of this study was to determine whether the larval nematode parasite, *Contracaecum* accumulates higher levels of metals than their host, *Schilbe intermedius* at two different dams in the Limpopo province (Flag Boshielo Dam, representing a moderate polluted site and Nwanedi-Luphephe Dam, representing a pristine site). This was achieved through the following objectives; determining the extent of selected metal bioaccumulation in the liver and muscle tissue of *S. intermedius,* determining metal levels of the nematode parasite, *Contracaecum* and correlating the host size with that of metal levels in the fish tissue and in the parasite.

Water samples were collected during March and April 2013 at both dams. Twenty silver catfish were collected between March and April 2013 from each dam by means of gill nets. The fish were weighed, measured (total, standard and fork length) and sacrificed by severing the spinal cord. Muscle and liver tissues were removed from each fish and the body cavity was opened to obtain the nematode larvae. The nematodes were cleaned in distilled water, counted and stored in glass bottles and frozen. The mean intensity, mean abundance and prevalence of *Contracaecum* were determined for possible correlation with host size and sex. Samples were sent to an accredited laboratory for detection (ICP MS) of selected metal levels which include: Ag, Al, As, B, Ba, Be, Bi, Cd, Co, Cr, Fe, Hg, Li, Mn, Mo, Ni, Sb, Se, Sr, Ti, U, V, Zn.

A prevalence of 100% was recorded for *Contracaecum* in silver catfish at both localities. The mean intensity for *Contracaecum* was higher for female than male fish. Higher levels for some metals were recorded in the parasite larvae than in fish tissue. Generally, higher levels of metals were recorded from larger fish. Furthermore, higher metal levels were recorded from fish at Flag Boshielo Dam than at Nwanedi-Luphephe Dam. The high mean intensity recorded at both sites made this parasite suitable for bioaccumulation studies.

Synodontella species (Monogenea: Ancyrocephalidae) from the gills of _Synodontis zambezensis_ peters, 1852 (Siluriformes: Mochokidae) in Flag Boshielo Dam, Olifants River, South Africa

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The gills of *Synodontis zambezensis* Peters, 1852 revealed the presence of two species of *Synodontella* (Monogenea: Ancyrocephalidae) in Flag Boshielo Dam, Olifants River, South Africa. Observations on the sclerotized structures of the specimens revealed two varieties with regard to the shape of the median extension and the lateral ends of the ventral bar. One species (*Synodontella* sp1) is characterized by the presence of a 'coiled' projection from the median extension with the ends of ventral bar a curved fork while the other (*Synodontella* sp2) is distinguished by the non-coiled projection of the median extension of the ventral bar and straight fork ends of the ventral bar. Descriptions and measurements of the two species are compared to other existing *Synodontella* species and a diagnostic key is suggested. These are the first records of Monogenea from this fish species in South Africa and their status as new species are revised.

Does parasite burden contribute to the poor condition of silver carp in Flag Boshielo Dam?

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The silver carp, *Hypophthalmichthys molitrix* (Valenciennes, 1844) which is indigenous to Asia (China) and Europe, is an invasive alien fish species found in most of the tropic and temperate regions of the world that includes South Africa. Silver carp were accidentally introduced in the early 1990's into Flag Boshielo Dam (24° 47′ 0″ S, 29° 25′ 40″ E) which is situated in the Olifants River System. This species has rapidly become a thriving feral population within the dam. However, over the past two years an increasing number of sporadic deaths of this species have been noted. It is for this reason that a total of 111 silver carp specimens, varying in size, were collected and examined (February 2012 to January 2013) in order to determine the health status of this species using the health assessment (HAI) protocol and parasites. Small sized carp were caught using conventional angling gear, while larger specimens were collected near the water surface using a scoop net. All ecto- and endoparasites collected were fixed and preserved according to standard methods. Of the 4 939 parasites retrieved, two ectoparasites and one endoparasite species were identified. Ectoparasites included one copepod, i.e. *Ergasilus* sp. found on the skin, fins and gills (prevalence 76%), and a branchiuran, i.e. *Argulus japonicus* on the skin and fins (prevalence 12%). The only endoparasite recorded was a digenean larva, i.e. *Diplostomum* sp. from the eye (prevalence 45%). Correlations between parasite infection and host size, as well as seasonal variation in their infection dynamics associated with seasonal changes, were detected and it was found that adult fish had higher infection levels compared to juveniles. Information obtained from this study is necessary to understand the effects these parasites have on host populations as well as expands the information on the rarely studied biology, epizootiology and ecological interactions of this species. In addition, knowledge on the parasites of silver carp when compared with indigenous fish species may give an indication of how adaptive these parasites are to new host species and local conditions.

Protopolystoma xenopodis (Monogenea, Polystomatidae) morphology

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Different stages of the life cycle of *Protopolystoma xenopodis* (Monogenea; Polystomatidae) strictly host-specific to the African Clawed Frog *Xenopus laevis* (Anura; Pipidae), were studied using scanning electron microscopy, histology and light microscopy. As the host is primarily aquatic there is no need to accumulate eggs *in utero*. Eggs are released continuously and are washed out when the frog urinates. After development of about 22 days an active swimming oncomiracidium leave the egg capsule and locate a potential post-metamorphic clawed frog. The oncomiracidium migrates to the kidney where it attaches and starts to feed on blood. Parasites then migrate to the urinary bladder where it reaches maturity. Eggs are fusiform, about 160 µm long and with a smooth surface and operculated. Oncomiracidia are elongated and cylindrical in shape, with an oval posterior cup-shaped haptor that bears a total of 20 sclerites; 16 marginal hooklets used for attachment to the kidney of the host and 2 pairs of hamulus primordia. Oncomiracidia has 64 discrete ciliated cells arranged in a consistent symmetrical pattern. The shapes of the ciliated cells vary in different regions: medial cells are often rounded whilst lateral cells are elongated and ellipsoidal. The cilia enable the oncomiracidium to swim for up to 24 hours when cilia curl up and become non-functional. Ciliated cells are shed from the body surface. The tegument between the ciliated cells bears a series of sensory papillae. Adult parasites' body broadens in the centre and narrows at the basis of the anterior mouth opening and posterior haptor. 16 marginal hooklets are no longer visible, and have been replaced by six large suckers and two large hamuli. Suckers are supple and assist in successful attachment to urinary tissue of its host. Body surface is free from ciliated cells or scar tissue as the parasite matures, but does still contain multiple sensillae.

Fine structure of *Hepatozoon* spp. gamonts and merogonic stages, parasitic in *Pseudocordylus melanotus* from Platberg in the Eastern Free State

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Haemogregarines have been described from several lizard families on most continents. In Africa, these intracytoplasmic infections were found and described only in a few lizard families, and in South Africa, only one species has been described in the endemic family of Cordylidae. Recently, the complete life cycle of this still unnamed species has been elucidated in the blood of *Pseudocordylus melanotus* and the final host (containing the sexual stages) was found to be a mosquito (*Culex* (*Afroculex*) *lineata*). Since the infective sporozoites reside in the gut of the mosquito, transmission of this infection to an uninfected host is achieved only by ingestion of an infected mosquito.

The objective this study is to show the key ultrastructural features of the gamont stages of this haemogregarine in the peripheral blood as well as the merogonic stages in the liver from an infected *P. melanotus* lizard. Blood samples containing gamont stages revealed specific apicomplexan structures through examination by transmission electron microscopy (TEM), and these structures included rhoptries, micronemes, golgi apparatus and dense granules. Gamont stages of infected host liver samples showed similar structures as above and the different developmental stages of meronts were surrounded by granular melanomacrophages, indicating an immune response from the host. It seems that these meronts were protected from destruction by the melanomacrophage cells by means of the parasitophorous vacuole and its limiting membrane, since no surrounding capsule was observed from the meronts.

The successful merogonic development in spite of the host immune responses are not completely understood, but could be partly due to the parasitophorous vacuole and the abundance of intracytoplasmic amylopectin within the different stages within the host's liver. These unique ultrastructural features thus confirm the identity of these haemogregarines as members of the genus *Hepatozoon.* This study shows the necessity of using ultrastructural features as a diagnostic tool and can aid in a better insight regarding their taxonomic status as well as the mode of transmission of these resilient parasites.

Ecological and morphological study of an unidentified microcotylid (Monogenea: Microcotylidae) on the gills of white stumpnose (*Rhabdosargus globiceps*) using both traditional morphology and molecular methods

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Monogeneans are important pathogens in aquaculture and have been responsible for significant losses in fish production. The sanguinivorous feeding of members of the monogenean family Microcotylidae are often associated with pathological to the host, which ultimately results in massive mortalities of cultured fish due to secondary infection by various micro-organisms. In South Africa, the white stumpnose, *Rhabdosargus globiceps*, is currently being evaluated as a potential candidate species for finfish aquaculture. A recent parasitological survey of wild caught white stumpnose revealed a relatively high abundance of an unidentified microcotylid. Thus, the overall parasite composition and vulnerability of white stumpnoise becomes a critical point of interest when assessing its viability as a potential aquaculture candidate species. The aim of the current study was therefore to identify the *Microcotyle* species found on white stumpnose using morphological classification and molecular techniques. This study also included the biology of the parasite to determine optimal environmental conditions for egg embryonation and to identify factors that are likely to enhance the hatching process of the egg. This data will provide the basis on which to predict the impact of this parasite on the viability of the white stumpnose in captivity. The ultimate objective would be to incorporate accurate knowledge of the diagnosis, impact, dynamics of the transmission and host-parasite interaction in the development of effective management strategies for this parasite on captive fish.

Differential expression in cattle- and buffalo-derived *Theileria parva* isolates

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Theileria parva, a tick-borne protozoan parasite, is the causative agent of cattle theileriosis known to occur in two forms, East Coast fever (ECF) and Corridor disease. Other than the clinical and epidemiological differences, there is no clear understanding as to why cattle-derived *T. parva* (ECF-causing parasite) and buffalo-derived *T. parva* (Corridor disease-causing parasite) infections cause different disease syndromes in cattle. To investigate this subject we have analysed transcriptomes from two *T. parva* isolates representing cattle- and buffalo-derived parasites. RNA isolated from lymphoblast cultures infected with the macro-schizont stage of two *T. parva* isolates, cattle-derived *T. parva* (Muguga) and buffalo-derived *T. parva* (7014) was subjected to sequencing using Illumina HiSeq™ 2000. A total of 3969 transcripts were successfully mapped to the *T. parva* genome sequence and of these 937 (23.63%) were differentially expressed (DE) [(FDR) ≤0.001 and Fold Change >2]. Analysis of DE transcripts showed that a majority (634, 68%) was up-regulated in *T. parva* (Muguga) and of these 49.5% have no known function. Of the 32% up-regulated in *T. parva* (7014), 44.5% also had no known function. The large number of down-regulated transcripts associated with intracellular membrane-bound organelles and localization processes suggests possible diminished biosynthesis and translocation of parasite proteins into the host cell in *T. parva* (7014) infections. Differential expression was also observed in 30 transcripts of the apicoplast, an organelle essential for parasite survival; 50% of these were up-regulated in *T. parva* (Muguga) including four transcription factors with an AP-2 domain, which are believed to play key roles in development and environmental stress response pathways. A range of genes including those belonging to three gene families, TashAT, TA9/TP9 and SVSP, have been proposed as candidate transforming genes. Among the DE transcripts identified in this study, 8 genes belonged to the TashAT family, 4 to the TA9/TP9 gene family and 38 belonged to the SVSP gene family. All 4 TA9/TP9 genes were down-regulated in *T. parva* (7014) along with 50 % of the TashAT genes and 97.4% genes of the SVSP gene family. Interestingly, 53 transcripts were found to be expressed only in *T. parva* (Muguga) while 44 were exclusively expressed in *T. parva* (7014). Most of the exclusively expressed genes had the RPKM value <10, suggesting low to medium expression. However, 11 highly expressed transcripts were identified in *T. parva* (Muguga) and only five in *T. parva* (7014). All five transcripts from *T. parva* (7014) were associated with the apicoplast. The functions of the majority of transcripts expressed exclusively in *T. parva* (Muguga) are currently unknown (hypothetical proteins), although two SVSP genes were identified. These findings demonstrate that more genes, including those implicated in host cell transformation, are up-regulated in *T. parva* (Muguga) than in *T. parva* (7014). Many hypothetical proteins were identified among DE transcripts, emphasising the need to identify their biological function in order to elucidate their molecular importance in the genetic diversity of *T. parva* parasites. These preliminary results suggest that proteins involved in parasite survival and the ability of the parasite to transform host cells may be the objects of further investigations into understanding the molecular dynamics of ECF and Corridor disease.

A SEM study of the micromorphology of the sucking louse *Linognathus angasi* from the nyala *Tragelaphus angasi*

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Nyala are a sought after game species for game ranching in the eastern bushveld areas of South Africa. High infestations of sucking lice may cause poor condition and anemia in domestic stock, and may similarly affect game. Sucking lice collected from wild nyala in the Ndumu Game Reserve in Kwazulu-Natal, were identified by light microscopy as *Linognathus angasi* Weisser & Ledger 1977. As the four *Linognathus* species found on the related bushbuck, nyala and greater kudu are morphologically very similar and difficult to distinguish, it was decided to do a scanning electron microscope (SEM) study to investigate the micromorphological characteristics of *L. angasi.* The lice preserved in 70% ethanol were ultrasonically cleaned, routinely prepared for SEM and viewed in a Zeiss SUPRA55VP FE-SEM.

Small scale-like setae covered the head, thorax and membranous abdomen. The post-antennal conical head bearing five pairs of setae, tapered to join the thorax while the short rounded pre-antennal part enclosed the flaps of the haustellum at the anterior tip**.** The antennal segments IV and V each bore a large pore organ with its sensory tufts, and two plate organs. The robust thorax bore a pair of large lateral spiracles and a pair of long setae on the mesothorax, and ventrally a linear sternal plate which is a distinguishing feature of *L. angasi*. Legs I each bore a slender claw while the claws of legs II and III were extremely robust closing against scaled distotibial pads for attachment. Abdominal segments II to VII bore large lateral spiracles while VI to VII were characterized by pairs of long marginal setae. The female gonopods VIII were curved medially each bearing a terminal fringe of setae while gonopods IX had a medial fringe of short setae. The abdomen of the male had a large genital plate that reached from segment VII posteriorly and terminated in a single bulbous process. The everted pseudopenis had a longitudinal keel-like ridge.

These micromorphological observations on *L. angasi* may contribute to the comparative taxonomy of the *Linognathus* species of the related Tragelaphine antelope.

Molecular detection of vector-borne haemoparasites in wild rodent species in South Africa

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It has long been recognized that wildlife can act as reservoirs for many human and livestock diseases and that arthropods are often involved in the transmission of pathogens. In recent years, vector-borne viral, bacterial and macro-parasitic diseases have emerged or re-emerged in many geographic regions causing global health and economic problems. The ecology and epidemiology of these diseases are affected by the interaction between the pathogen, the host and the vector, with the addition of the environment in certain cases and is thus of major concern. In particular, rodents often harbour pathogens and parasites that can affect humans. To date little is known with regard to pathogens that naturally occur in rodents in South Africa and thus no information is available of the risk to livestock and/or humans. The aims of the study were, to record the pathogen diversity within wild rodent species and to establish the risk of tick-borne haemoparasitic diseases to livestock and humans. DNA was extracted from 423 blood samples collected from 5 rodent species (*Rhabdomys pumilio*, *Otomys* sp., *Otomys irroratus, Micaelomys namaquensis* and *Myotomys unisulcatus*) and 1 insectivore (*Crocidura* sp.). The animals were trapped in 5 provinces (Western Cape, Northern Cape, Eastern Cape, Free State and KwaZulu Natal) in South Africa and were screened for the presence of *Theileria, Babesia, Ehrlichia* and *Anaplasma* spp. using the Reverse Line Blot (RLB) hybridization assay. Results obtained revealed the presence of *Babesia microti, Anaplasma bovis* and *Ehrlichia ruminantium,* either as single or as mixed infections. A major finding is the presence of *Babesia microti* and *Ehrlichia ruminantium* in the rodent samples*. Babesia microti* is known to cause human babesiosis; a zoonotic, malaria-like illness that can be particularly severe and sometimes fatal in elderly, asplenic, or immunecomprized persons. It has not been reported in South Africa before and this finding is of public health concern and should be confirmed using cloning and sequencing. The presence of *E. ruminantium* would suggest that these rodent species could act as natural reservoir of heartwater infection and could play an important role in the epidemiology and spread of the disease, which may represent a serious threat to the livestock industry. 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, but only with the genus-specific probe, suggesting the presence of novel species or variants of a species. These samples are currently under investigation.

Morphological and molecular characterization of Diplostomid metacercariae (Digenea: Diplostomatidae) from freshwater fishes in the Tshwane area, Gauteng Province, South Africa

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The larval stages of diplostomid digeneans inhabit freshwater fish species as their second intermediate hosts. The classification of these parasitic stages to the species level using only morphology is very challenging due to the lack of genitalia that are regarded as the most important structures in the identification of these organisms. In addition to morphological descriptions, this pilot study was aimed at assessing the possibility of applying molecular biology techniques to the investigation of larval diplostomid parasites. Diplostomid metacercariae were collected through dissections of three fish species. Standard techniques of light and scanning electron microscopy (SEM) were employed to study the internal and external ultrastructures of these parasites. Polymerase chain reaction (PCR) techniques were also performed pertaining to the molecular structures on the same species. The diplostomid metacercaria inhabiting the brain cavity of *C. gariepinus* was morphologically identified as *Diplostomulum (Tylodelphys) mashonense*. An unknown *Diplostomulum* was found unencysted in the vitreous chamber of *P. philander* and *T. sparrmanii*. Both parasitic species' 28S rDNA genomic region was successfully amplified using Dig 125/1500R primer pairs. The assay yielded a product of 1300bp as seen on the gel images. There were 13 nucleotide differences over the entire analysed sequences resulting in a 0.55% DNA differences. From the 1273bp of a 28S region, 1.03% difference exists at DNA level. In line with the morphological characteristics of these parasites, there seems to be slight difference in their genetic make-up. The application of molecular techniques on digenetic trematodes seems very promising and may yield great potential in future descriptions of morphologically similar parasitic species.