Parasitological Society of Southern Africa

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Keynote Addresses

A lousy litany

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This presentation focuses on lice collected from wildlife and Angora goats examined at regular intervals in diverse regions of South Africa. Louse species composition on hosts of the same species varied with locality. The species proportions of louse populations on young animals differed from those on older animals. Drought-affected animals had larger louse burdens than unaffected animals. Males had significantly higher or lower louse and other ectoparasite burdens than females of the same species. Sets of blue wildebeest, impalas and Angora goats were examined at monthly intervals from shortly after birth until they were 2 years old. The seasonalities of *Damalinia theileri* and *Linognathus gorgonus* on blue wildebeest were similar in the 1st year of their lives, but differed in the 2nd year. A gastro-intestinal nematode exhibited a remarkably similar seasonality to that of *D. theileri.* Significantly more foot lice, *Linognathus nevilli,* were present on impala lambs than on yearlings. At 1 week of age Angora goat kids were already infested with small numbers of *Damalinia limbata* and *Linognathus africanus,* and harboured 1000s 3 weeks later. *D. limbata* was not affected by the goats' age, but its numbers were drastically reduced by shearing, whereas *L. africanus* appeared to be a parasite of goat kids and perhaps heavily pregnant ewes. A seasonal peak in the numbers of *Linognathus oryx* on gemsbok may have been delayed by co-infestation with large numbers of ticks. Burdens of *Haematopinus phacochoeri* on warthogs peaked from winter to spring and consisted largely of nymphs.

The impact of cross-border initiatives on malaria control

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Although not widely spread throughout the country, malaria still remains one of the most important diseases of public health importance in South and southern Africa. For a long time malaria was viewed as a country specific disease but recent trends in disease patterns have indicated that malaria is best viewed as a disease of regional importance. Therefore, to make further advances towards malaria control in South Africa, the Lubombo Spatial Development Initiative (LSDI) was initiated in the high malaria areas of Mozambique, South Africa and Swaziland. The aim was to strengthen malaria control efforts in this area through shared resources and expertise. Spraying with residual insecticides was introduced in a sustained manner. Prevalence rates were one of the main indicators used to monitor the impact of the spray programmes in the LSDI areas. A secondary indicator was vector density, which was measured continually at household level. In the past 10 years, the LSDI programme has had a marked impact on disease transmission in the region. Compared to a baseline year of 2000, malaria incidence in South Africa has decreased by 99 % in KwaZulu-Natal, 99 % in Swaziland and the prevalence in Mozambique has decreased by 96 %. The success of this initiative was demonstrated by the SADC Ministers of Health endorsing the elimination of malaria from South Africa and Swaziland by 2018. Furthermore, other initiatives modelled on the LSDI have been implemented in other parts of southern Africa.

Parasites as biological tags in fish population studies

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The use of parasites as biological tags in fish population studies is reviewed. The method dates from 1939, since when it has been used with increasing frequency. The history of parasite tagging is presented, the changing criteria and guidelines applied to the selection of tag parasites are given, and landmark publications are highlighted. The advantages and disadvantages of parasite tags compared with other methods used in fish population studies, such as host genetics and mechanical tagging, are discussed. Recent trends and developments such as the use of molecular methods and the application of multivariate analyses are described. Parasite tags are most effective when used in combination with other tagging methods in multidisciplinary projects and some successful recent studies are described to illustrate this point. Finally, the potential of parasite tags for stock identification of South African sardines is examined.

Oral Presentations

Digestive system morphology in Branchiura, their feeding and the damage inflicted

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The Branchiura are parasites of fishes. In *Argulus* an extended proboscis and pre-oral spine occur. Digestive enzymes are released *via* the pre-oral and the labial spines from glands situated in the carapace. A blood meal is taken when blood vessels are broken. In *Dolops* and *Chonopeltis* the proboscis is very short and both the pre-oral spine and oral spines are absent. *Dolops* is able to feed on blood following its release from the wounds created by the hooks on the maxillulae. In *Chonopeltis* attachment is effected by suckers, and mucus forms the main component of the diet. In all 3 genera the oesophagus is surrounded by circular muscle bundles that extend the oesophagus to create a vacuum, presumably to assist ingestion. The oesophagus opens into the midgut *via* a funnel. Superficially the anterior and posterior midgut in all 3 genera are similar. In *Argulus* and *Dolops* the ultrastructure is similar to that of free-living copepods. The anterior midgut consists of resorptive cells and the enteral diverticula contain both F (fibril) and R cells. The R cells contain oil droplets and the F cells contain rough endoplasmic reticulum indicating that absorption takes place in the anterior midgut and enteral diverticula. Blister cells occur in the posterior midgut and are involved in processing of digestive waste. In *Chonopeltis* R/F cells occur with both absorption and digestive waste involvement. The cells contain few lipid droplets but instead have crystalline protein structures. The difference in diet is thought to be influenced by the feeding appendages and the ultrastructure of the digestive system, which impacts on the pathology exhibited by the parasites.

Description of *Babesia lengau* infecting cheetah from southern Africa

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In a previous study, we reported on a large number of cheetah blood specimens that gave positive signals only for *Babesia* and/or

Theileria genus-specific probes on the reverse line blot (RLB) assay, indicating the presence of a novel species or variant of existing species. Some of these specimens were investigated further by microscopic, serological, sequencing and phylogenetic analysis. The near full-length 18S rRNA gene as well as the 2nd internal transcribed spacer (ITS2) region of 13 samples were amplified, cloned and sequenced. A species-specific RLB probe, designed in the hypervariable V4 region of the 18S rRNA gene to detect the novel *Babesia* sp., was used to screen an additional 137 cheetah blood specimens for the presence of the species. The prevalence of infection was 28.5 %. Here we describe the morphology and phylogenetic relationships of the novel species, which we have named *Babesia lengau* Bosman, Oosthuizen, Peirce, Venter and Penzhorn, 2010.

Combined use of fluorescence resonance energy transfer (FRET) and real-time PCR to discriminate between 6 *Theileria* species in cattle and buffalo

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Theileria parva is the causative agent of cattle theileriosis in East and southern Africa. It is transmitted by ticks of the genus *Rhipicephalus* and African buffalo are healthy carriers. It usually co-occurs with non-pathogenic *Theileria* species in infected buffalo, and although these parasites do not have any economic significance, they can interfere with the diagnosis of *T. parva.* Diagnostic methods used for the detection of *T. parva* should therefore be sensitive and specific to ensure accurate diagnosis. A real-time PCR assay based on the 18S ribosomal RNA (18S rRNA) gene was developed and is currently used for the diagnosis of *T. parva* in cattle and buffalo in South Africa. However, the presence of *Theileria* sp. (buffalo) in mixed infections can compromise the specificity of this assay. More recently, a nested fluorescence resonance energy transfer (FRET) real-time PCR based on the cytochrome oxidase subunit (CoxIII) gene was developed and used for simultaneous detection and differentiation, by melting curve analysis, of 6 *Theileria* spp. from cattle samples using the Rotor Gene 3000 (Corbett Research). The assay was further modified to be able to detect *Theileria* sp. (buffalo). The aim of the study was to evaluate the modified assay in the identification and differentiation of the *Theileria* spp. of the African buffalo. Two hundred and twenty-four buffalo and cattle samples from different localities in South Africa and Mozambique were analysed. DNA was extracted and subjected to the nested FRET real-time PCR and RLB assays. Positive controls of CoxIII genes of reference *Theileria* spp. and a negative control were included in each run. Primary PCR products of the CoxIII gene of 10 selected samples were cloned, sequenced and the sequences analysed. *Theileria parva* and *Theileria* sp. (buffalo) were the most commonly detected species in both assays. The real-time assay did not detect *Theileria velifera* in any of the samples, and 4.5 % of the samples were negative or below detection limit. The RLB detected more infections of *T. mutans. T. buffeli* and *T. velifera* than the real-time PCR assay. *T. taurotragi* infections were not detected by the RLB. Unexpected peaks were obtained from some buffalo samples that were either between the peaks of *T. parva* and *T. velifera, T. mutans* and *T. buffeli*, or *T. buffeli* and *T. taurotragi.* Primary PCR products from some of these samples were cloned and sequenced. In total 27 new sequences were obtained. Sequence data revealed extensive sequence variation in the gene between and within species. The coxIII real-time PCR assay is more sensitive than the RLB assay in the detection of *T. parva* and *Theileria* sp. (buffalo) but less sensitive in the detection of the other *Theileria* spp. of buffalo. Although the new test accurately detects and differentiates 6 *Theileria* spp. in cattle, the extensive variation in the Cox III gene of buffalo *Theileria* spp. compromises the specificity of the test in detecting infections in buffalo. The new sequences can be used to develop new primers and probes to increase the specificity of the test.

Parasite succession on marine fish in a pilot scale sea cage culture system in South Africa

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The development of a marine finfish aquaculture industry in South Africa is largely constrained by the availability of suitable coastline for fish farming operations. The 'high energy' South African coastline has necessitated that most of the development in this industry currently relies on pump-ashore, land-based systems for both hatchery and on-growing operations. Current industry growth and diversification has, however, led to the development of a pilot scale sea cage project in the Port Elizabeth harbour. The impacts of parasites and other pathogens associated with fish in sea cages have been well documented. To determine the rate at which parasite populations establish on newly introduced, uninfected fish in sea cages, a complete health examination was conducted on each batch of fish prior to stocking them into the sea cages. Monthly gill sets were obtained from 10 fish in each cage and quarterly histology samples were taken from full organ sets of fish from each cage. Monogeneans, particularly *Sciaenicotyle sciaenicola* from *Argyrosomus japonicus* and *A. inodorus* and *Zeuxapta seriolae* from *Seriola lalandi* were found to be most prevalent and pathogenic. The component population growth rate of *S. sciaenicola* was much higher than that for *Z. seriolae,* possibly due to high infection pressure from the resident *Argyrosomus* species in the vicinity of the cages.

Aspects of the biology of *Ergasilus* from the skin of freshwater fishes

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The 1st African member of the family Ergasilidae was described in 1909 by Sars, who found 3 species of *Ergasiloides* (Sars) in Lake Tanganyika. These have since been synonymised with *Ergasilus.* Fifteen species of *Ergasilus* occur in Africa on gills of freshwater fishes. Recently several *Ergasilus* specimens were found on the skin of fishes. Previously, in 2 instances unidentified *Ergasilus* specimens were recorded on skin. Specimens for the current study were collected using gill nets in Flag Boshielo Dam and Vaal Dam and investigated for copepod infections. The collected parasites were fixed, preserved in 70 % ethanol and studied with light and scanning electron microscopy. The morphology was compared with that of African *Ergasilus* spp. and it was found to differ from all known spp. in its unique antennular structure. It is also different from *Neoergasilus japonicus* previously described from the skin of fish. Antennules of *Ergasilus* species are 4-segmented while those of *N. japonicus* are 6-segmented. During 4 surveys, *Ergasilus* sp. exhibited a prevalence ranging between 21 and 75 %, mean intensity ranging from 1.3 to 1.9 individuals per host and mean abundance 0.331.27. *Ergasilus* sp. thus exhibited an aggregated distribution in some surveys and a random distribution in the others.

Effects of climatic variables and anthropogenic habitat change on the gastrointestinal parasite burden and immune genes (MHC) of the striped mouse, *Rhabdomys pumilio*

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As a consequence of environmental change, it is expected that shifts in temperature and precipitation patterns will influence para-

site communities and their hosts. Only minor changes in parasite distributions may expose new host populations lacking appropriate immunity or facilitate new contact zones between wildlife and domestic animals, livestock and humans. Understanding the interplays between climate variables, anthropogenic habitat change and gastrointestinal parasite burdens as well as adaptive immune gene variability (MHC) is fundamental to provide key insights into the factors shaping appearance, spread and distribution of diseases. We used the striped mouse, *Rhabdomys pumilio,* as a model and trapped 470 mice along a natural precipitation gradient from the Cape of South Africa to northern Namibia. Faecal egg counts of 439 sampled individuals and dissections of 161 gastro-intestinal tracts have been analysed. Our study revealed a significant positive correlation between mean annual precipitation and nematode infestation rates of animals and a negative correlation with temperature. In addition, we detected associations between precipitation and different qualitative measurements of parasite burden (mean nematode species richness, mean number of nematode worms and infection intensity per individual host). Owing to differences in parasite selection pressure across the gradient, hosts showed natural adaptations at the adaptive MHC genes. This is the 1st study that confirmed interactions between climatic variables, parasite infestation and adaptive immune genes in a free-ranging southern African small mammal. Further investigations will examine the effect of anthropogenic habitat change on helminth burden in the Western Cape Province.

The development, optimisation and evaluation of DNA-based molecular methods used to diagnose abalone tubercle mycosis (ATM) caused by *Halioticida noduliformans* in South African abalone, *Haliotis midae*

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Land-based abalone aquaculture in South Africa, based on the local species *Haliotis midae,* started in the early 1990s and has grown rapidly in the last decade. This industry is currently threatened by a fungal disease called abalone tubercle myscosis caused by a Peronosporomycete (formally Oomycete), *Halioticida noduliformans.* Diagnosis of this disease is based on the observation of gross clinical signs and histopathology. These methods fail to be sensitive enough to identify the causative agent accurately and reliably. Molecular confirmation could provide quicker, more accurate diagnostic information. This study aimed to develop a DNA based diagnostic test for the detection and identification of *H. noduliformans* in abalone. Nucleotide sequences of the small- and large-subunit rRNA and mitochondrial cytochrome oxidase subunit II (cox2) genes of *H. noduliformans* were compared with closely related Peronosporomycete gene sequences to identify potential polymerase chain reaction (PCR) primer sets. *H. noduliformans* specific PCR and real-time PCR primer sets were designed and optimised for each of the selected genes. Results indicate that, although all tested primers sets could amplify fungal DNA, only the LSU rRNA gene PCR primer set demonstrated no cross-amplification with closely related Peronosporomycete and non-fungal DNA. DNA extraction protocols were optimised to insure efficient and repeatable extraction of high quality fungus DNA from pure fungus and infected tissue samples. PCR amplification efficiency and potential inhibition were examined for each extraction method. Our preliminary results suggest that real-time PCR has great potential for monitoring and quantifying *H. noduliformans* on abalone culture facilities in South Africa.

Behavioural influence of eye flukes (Diplostomidae) on fish hosts

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Fieldwork carried out in the Okavango and Orange River Systems has indicated that the eyes and brains of a large variety of fish species are infected with larval digenetic trematodes belonging to the family Diplostomidae Poirer, 1886. In order to complete the life cycle, these endoparasites need to be transmitted to a final host such as a piscivorous bird. The probability that eye flukes could impair the fishes' escape response, by sight impairment, would lead to an increase in these parasites' transmission success. Histological sections of the eyes of infected *Tilapia* spp. have indicated the presence of either retinal or choroid detachments, possibly causing total/partial blindness or a distorted view, thereby rendering the fish host more susceptible to aerial predation. These detachments are likely the effect of blood-flow obstructions caused by the trematode cysts, which are lodged in the eye blood capillaries. Behavioural experiments to date indicated that infected *Tilapia* specimens were still able to detect movement of an aerial predator flying overhead, which diminishes the possibility of total blindness due to complete retinal detachment. In order to determine the possible behavioural effects of more localised retinal or choroid detachments, the ability of infected *Tilapia* to detect change in light intensities was determined. Individual fish were kept in small aquaria in a dark room and exposed to light flashes of different intensities, while their behavioural responses were documented. Preliminary results indicate a positive correlation between light intensity and behavioural response intensity, where infected *Tilapia* specimens were able to detect a light intensity of as little as 20 %. This suggests that diplostomatid eye infections have no obvious altering effect on the intermediate hosts' behaviour, under natural conditions.

Delicate *versus* robust: a comparative study on *Trichodina heterodentata* found on fish and tadpoles in southern Africa

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Trichodinids (Ciliophora: Peritrichia) inhabit a diverse range of aquatic habitats. Some are found associated with marine fish, others with salamanders, crustaceans or as in the case of *Trichodina pediculus* on hydra species as well as fish. Six trichodinid species are found on the skin and gills of certain juvenile anurans worldwide. One of these, *T. heterodentata,* is found on *Xenopus laevis* and *Amietophrynus* spp. in southern Africa. *Trichodina heterodentata* is globally distributed and is commonly found on cichlid fishes. Previous studies found that this particular trichodinid had delicate, thin rays when associated with tadpoles, in comparison to those found on fish, where rays were more robust. To date, most trichodinid studies concentrate mainly on their taxonomy, especially re-investigating or re-describing known species by using modern techniques. One such new method is proposed by Gong and his co-workers, where the areas of the blade, central part and ray of a single denticle are calculated and then repeated for every 3rd denticle. The averages of these are used to calculate the relevant total area for the specified trichodinid species. The results for different species are then compared. In the current study, *T. heterodentata* specimens from tadpoles and fishes were used to determine whether a modified version of Gong's method may aid in determining 2 options: firstly, do the populations from anurans represent a different (and therefore a new) species to those from fish, or secondly, if anurans and fish both harbour *T. heterodentata,* can this method indicate any distinct differences between these 2 host groups?

Aspects of the damage caused by the attachment clamps of a *Diplozoon* sp. on *Labeo umbratus*

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Labeo umbratus is a bottom-dwelling fish that feeds on soft sediment and detritus. They prefer still or slow moving waters, which are the same conditions preferred by the *Diplozoon* species. The *Diplozoon* species are blood feeding parasites found on the gills of

fresh water cyprinid fish. They attach to the primary lamellae of the gills by squeezing the gill epithelia with their clamps. They move around on the gill surface by alternating attachment and release of the anterior and posterior attachment organs; they secrete an adhesive substance to facilitate temporary attachment. The reproductive stage can only be reached after the fusion of 2 hermaphroditic diporpa in a permanent cross copula. Fish body size, age and stress are positively associated with the prevalence and intensity of the parasite on the gills. The attachment process is stimulated by chemical signals and the presence of the correct host structure (gills). There are 2 sets of attachment structures, namely the anterior and posterior attachment organs. The anterior structures consist of 1 pair of suckers and a gland that secretes a sticky adhesive substance. The posterior structures consist of 4 pairs of clamps on each opisthaptor and 1 pair of hooks. These posterior attachment structures can be further subdivided into 2 groups, namely scleride structures and parenchymatous structures. The clamps are supported by a series of scleride rods which maintain the shape, rigidity and strength of the clamps. The samples were collected at the Vaal Dam and then processed according to standard histological procedures. Scanning electron and light microscopy were used to study the clamp morphology. Minimal pathology was observed; there was compression of the epidermal tissue and secondary lamellae. Haemorrhaging and excessive mucus production was also evident in the histological sections. The *Diplozoon* species attachment causes little if any damage to the host although they obtain their nourishment from feeding on blood.

Metazoan parasites of *Labeo rosae* from Flag Boshielo Dam, Olifants River System, Limpopo Province

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The Olifants River is 1 of the most polluted rivers in South Africa due to mining, industrial and agricultural activities. The 1st (Flag Boshielo Dam) and the last (Phalaborwa Barrage) impoundments along this river in Limpopo Province are being biomonitored and compared for the impact of water and sediment quality on the health of fish. The present study on parasites of *Labeo rosae* forms part of this umbrella project on the Health Assessment Index (HAI) which includes the Parasite Index (PI) of several angling, fisheries and important food fish species in the 2 impoundments. During seasonal surveys conducted from July 2009 to June 2010, 40 fish specimens were examined using the HAI and routine parasitological methods. Of the 144 parasites retrieved, 7 species were ectoparasites and 2 endoparasites. Ectoparasites found were *Dactylogyrus pienaari, Dactylogyrus* sp., *Dogielius* sp., *Diplozoon* sp., *Lamproglena* sp., *Lernaea cyprinacea* and *Ergasilus* sp.; endoparasites include *Paracamallanus cyathopharynx* and *Nematobothrium* sp. The 2 *Dactylogyrus* spp. and *Dogielius* sp. are being investigated as new species, while *Nematobothrium* sp. and *Ergasilus* sp. are new records for southern Africa for *L. rosae.* The study will make people aware of the parasites that might be a health risk when consuming fish. Furthermore, the dam is frequently used for angling competitions with a positive impact on local tourism and economy. This project should impact on the management and conservation of water in the Olifants River and thus in Limpopo Province.

Establishment of *Babesia gibsoni* antigen slides and control sera for the indirect fluorescent antibody test

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Babesia gibsoni, a haemoprotozoal parasite transmitted by ticks, has not yet been reported in South Africa. Owing to the increased international travel and migration of people and their pets, concerns for its introduction and epidemics of canine babesiosis have arisen. It is, therefore, vital that specific and reliable diagnostic tests are available for early detection of this disease. A 2-year-old male dog was experimentally infected with *B. gibsoni* to produce antigen slides and control sera for the IFA test. The disease progression was monitored by taking body temperature, packed cell volume (PCV) and level of parasitaemia. Blood stabilates were prepared when the piroplasm counts were high and stored for future reference purposes. Blood for serum was collected from the dog before infection and at regular 2-weekly intervals after infection. The dog's temperature appeared not to be affected by *B. gibsoni* infection, but a high correlation was seen between PCV and parasitaemia, where PCV decreased gradually as the percentage of infected RBC increased. The PCV dropped from 45 to 12 % while the parasitaemia reached 30 %. The antibodies were 1st detected on day 66 post infection and positive serum was collected. The dog was given a blood transfusion and a single dose of imidocarb dipropionate on days 38 and 62 post infection and by day 80 post infection it was euthanased. Antigen slides were prepared for the IFA test, analysed and standardised to correlate with official OIE requirements for the diagnosis of *B. gibsoni.*

Detection of canine *Babesia* and *Ehrlichia* infections from blood and tick samples of domestic dogs in the eastern Free State Province, South Africa

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The aim of this study was to document information about ticks and tick-borne diseases of domestic dogs in the eastern Free State Province of South Africa, particularly targeting those in rural areas that do not receive veterinary attention. A total of 105 (n = 91 ticks and n = 14 blood dried on FTA cards) samples were collected from domestic dogs in Qwaqwa, Kestell and Harrismith in the eastern Free State Province. All samples were screened for the presence of canine *Babesia* and *Ehrlichia* species using conventional PCR assays amplifying specific fragments located between 18S rRNA–28S rRNA for canine *Babesia* parasites and 16S rRNA for *E. canis.* Results indicated that 2/105 (1.9 %), 9/105 (8.57 %) and 29/105 (27.6) were PCR positive for the presence *Babesia rossi*, *B. vogeli* and *E. canis* infections, respectively. Although the majority of ticks identified were *Haemaphysalis elliptica,* low numbers of *Rhipicephalus* species were also found infesting sampled dogs.

Diversity of metazoan parasites of the Mozambique tilapia, *Oreochromis mossambicus,* as indicators of pollution in the Limpopo and Olifants river system

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To establish the relationship between metazoan parasite communities and water quality levels, the present study examined the fish metazoan parasite communities of 3 dams with varying degrees of pollution: Nwanedi-Luphephe, Flag Boshielo and a Return Water Dam at a mine site. The Mozambique tilapia, *Oreochromis mossambicus,* was used as a model host. Seasonal sampling of fish for parasitological examination was done at all sites from January 2008 to January 2010. Water samples were taken concurrently and selected variables determined. The parasite assemblage comprised 20 species in total. There was a significant decrease in species richness and abundance values of parasites at the severely polluted site. The distribution of the branchiuran *Dolops ranarum* and the digenean T*ylodelphys* was limited to the unpolluted site. The monogeneans

and nematodes decreased along the pollution gradient, showing their sensitivity to organic pollution. The digenean *Diplostomum* type 3 sp. was the most tolerant and occurred at all the sites. The gryporhynchid cestode larvae were most abundant at the severely polluted site, probably as a result of high abundance of oligochaetes and copepods, their intermediate hosts that thrive in nutrient-enriched sediments, at the mine site. The variability of the calculated biotic indices (Shannon, Evenness) and the degree of interactivity among parasites suggests that the structure of parasite communities is affected by water contamination levels. The observed results assume that the decrease in parasite diversity can be related to increased organic pollution. Some parasites with complex life histories were absent in association with pollution-related disappearance of their vector hosts.

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

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Babesia rossi-induced canine babesiosis is associated with severe clinical manifestations and mortalities. Further information is required regarding the relationship between parasite genotype and disease phenotype. The current techniques of genotyping include: PCR, RLB (RLB primers) 2nd PCR with different primers, sequencing, sequence analysis and phylogenetic analysis. These methods are time consuming. In this study, a highly sensitive real-time polymerase chain reaction (PCR) assay was developed to detect *Babesia rossi* genotype in dogs. A total of 106 blood samples was obtained from *B. rossi*-infected dogs at Onderstepoort Veterinary Academic Hospital (OVAH). Samples were screened for the presence of parasites in DNA using the reverse line blot (RLB) hybridisation assay. Positive *B. rossi* samples were selected and the BrEMA1 gene was amplified and sequenced using FrepBrEMA1 (5′-CCA ACA TTG ATG ATG ACA A-3′) and RrepBrEMA1 (5′-CTG CAT GTC AGC TTA ATC A-3′) primers. These primers were also used for a real-time PCR to specifically amplify the BrEMA1 gene using SYBR Green. Although successful in detecting BrEMA1 genotype using SYBR Green, the assay failed to reliably differentiate amongst the various genotypes. A new approach using hybridisation probes is currently being explored. Based on sequence analysis, we have identified 9 genotypes in agreement with previously published results and the existence of an additional 3 new genotypes.

Diversity in *Theileria* from cattle and Cape buffalo (*Syncerus caffer*)

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Sequence variation within the 18S SSU rRNA V4 hyper-variable region can affect the accuracy of real-time hybridisation probe based diagnostics for the detection of *Theileria* spp. infections. Differences occurring in this region within- or between species need to be assessed when diagnostic assays are designed and validated. The *Theileria* 18S SSU RNA sequences from ~100 cattle and Cape buffalo were cloned, sequenced and analysed. Approximately 1000 clones were sequenced and 17 unique genotypes detected. These included 4 *T. buffeli*, 5 *T. mutans*, 3e *T. velifera* and a *T.* sp. sable-related genotype. *T. parva* was found in both cattle and buffalo and 7 variants were detected. *T.* sp. (buffalo) and *T.* sp. (bougasvlei) were detected in buffalo and *T. taurotragi* in cattle. Buffalo possessed a larger variety of genotypes and more genotypes per animal compared to cattle. Comparison with reverse line blot indicated that sequencing detected more genotypes. The results indicate a high level of *Theileria* diversity in cattle and buffalo of southern African origin. Analysis by real-time PCR indicates that the genotypes detected do not affect detection by the 18S hybridisation probes.

Testing for piroplasms – how this affects the equine industry

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In South Africa, 1619 horses were sold during 2008/2009 for more than R250 000 000. Thoroughbreds exported during 2008/2009 totalled 51 and 385 horses were imported. The presence of piroplasms in equines at the time of sale, importation or export directly influences movement status, price, and also negatively affects equine performance The ARC-Onderstepoort Veterinary Institute, Parasites, Vectors and Vector-borne Diseases Programme (PVVD) uses the Indirect Fluorescent Antibody Test (IFAT) as a routine diagnostic tool for testing for antibodies against *Theileria equi* and *Babesia caballi*. A major client submits samples for testing for the National Yearling Sales (NYS), National Two Yearling Old (N2YO) and Ready To Run (R2R) Sales. For the N2YO 2008 sale, 24 % of the horses were seropositive for *T. equi* and 9 % for *B. caballi* and for the R2R, 3 % were seropositive for both *T. equi* and *B. caballi*. For the NYS 2009 sale, 22 % and 4 % of the horses were seropositive for *T. equi* and *B. caballi*, respectively, 34 % and 11 % for the N2YO 2009 sale and 9 % and 3 % for the R2R 2009 sale. For the NYS 2010 sale, 18 % and 0.4 % of horses were seropositive for *T. equi* and *B. caballi*, respectively, while the N2YO 2010 sale showed 33 % and 13 % seropositive. Tests are also done for international clients. IFAT antigen slides are produced using tissue culture and are available for sale to other laboratories.

Rodents in fragmented habitats: the effect on rodent body size and disease risk to humans and domestic animals

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Agricultural development is an important cause of losses in plant and animal diversity, driven at least in part by habitat fragmentation. However, for the opportunistic rodent, farming practices may be beneficial by making additional food and protection available. To date few studies, and none in South Africa, have tested whether fragmentation has an effect on the macroparasites that occur on vertebrate hosts. The regionally widespread striped mouse, *Rhabdomys pumilio*, was live-trapped at 6 localities (3 pairs of pristine natural areas and habitat fragments) in the Cape Floristic Region during 2003 and 2004. Sex, reproductive state and body measurements were recorded for each animal. All ectoparasites were removed, counted and identified to species level. Generalised linear models were used to record whether fragmentation had an effect on parasite species richness and abundance. There was no significant difference in the species richness between natural and fragmented sites for all the parasites together or for the individual tick, mite and flea taxa. Parasite abundance was significantly higher in fragments compared to natural areas for all the parasites together. Considering taxa separately, this pattern was recorded for ticks and also for the 3 most abundant individual tick species and 1 of the most abundant flea species. In contrast, a significantly higher abundance was recorded for fleas in the natural areas compared with fragments. Intraspecific comparisons showed that 2 of the 3 most abundant fleas also displayed this pattern. Parasite species composition differed significantly between the natural and fragmented areas, although only a small proportion of the variance in species composition was attributed to fragmentation.

Spatial distribution of three stages of *Diplectanum oliveri* on the gills of *Argyrosomus japonicus* (dusky kob)

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Monogeneans typically have direct life cycles and are generally

considered to be host- and site-specific. This site specificity or microhabitat preference particularly among the gill-inhabiting Dactylogyridea has been well documented and often ascribed to water currents, space and in the case of mixed infections, interspecific competition for resources. This study was conducted to investigate spatial distribution of the postoncomiracidium, pre-adult and adult stages of the monogenean *Diplectanum oliveri* on the gills of *Argyrosomus japonicus.* For this evaluation, the gills were divided into left and right and the gill arches were numbered from 1 to 4, 1 being the nearest to the operculum. Each gill arch was then further subdivided into 12 partitions. The infection of *D. oliveri* on *Argyrosomus japonicus* was found to be symmetrical between left and right, but preferences for specific gill arches and subdivisions of each hemibranch by all 3 stages were found. These findings are interpreted with respect to onchomiracidial recruitment to the gills of the host and the metamorphosis of the opisthaptor and attachment to the host.

A comparative study among the diplozoids (Monogenea, Diplozoinae) of southern Africa

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The family Diplozoidae Yamaguti, 1963 comprises host-specific worms found on the gills of cyprinid fish. Diplozoids are the only members of the Monogenea where 2 hermaphroditic adults fuse and live in permanent copula. Little is known of the African Diplozoidae fauna and even less of the southern African fauna, especially when compared to the European and Asian fauna. To date 2 species of *Diplozoon* von Nordman, 1832 are known from Northern Africa, *i.e. D. aegyptensis* Fischtal & Kuntz, 1963 collected from *Labeo* spp and *D. ghanense* Thomas, 1957 described from *Alestes* spp. *Afrodiplozoon polycotyleus* Paperna, 1973, previously of the genus *Neodiplozoon* Tripathi 1959, was described from *Labeo victorianus* in Kenya and from *Barbus* spp. in Uganda and South Africa, as well as an undescribed species from the genus *Paradiplozoon* Achmerov, 1974, collected from *Labeobarbus aeneus.* Members of the Diplozoidae were collected from 9 cyprinid fish species during fish parasitological surveys over the last 15 years from the Okavango, Orange-Vaal and Zambesi River Systems. Identification of species is mainly done on the basis of the length of the central hook, the shape of clamp sclerites and host specificity. The collected specimens have various morphological differences such as the attachment clamps situated on the opisthaptor, which range from 4 to as many as 11 pairs. Three different species have provisionally been identified belonging to the genera *Diplozoon, Paradiplozoon* and *Afrodiplozoon.* The aim of this study is to investigate the differences among these species, focusing on morphological differences of especially the attachment clamps, while also shedding light on the method of attachment and host specificity, as well as parasitehost interaction.

Protein gene candidates for the molecular detection of *Theilera parva* in African buffalo (*Syncerus caffer*) from southern Africa

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The real-time hybridisation PCR assay targeting the 18S SSU rRNA V4 hyper-variable region of *Theilera parva* can be affected by mixed infections with *T.* sp. (buffalo)-like parasites. Therefore, molecular markers independent of the 18S rRNA gene were investigated for diagnostic potential. This included the p67, p104 and Tpr genes that are unique to *T. parva.* A conventional and SYBR® Green touch-down PCR method was developed for each gene and 240 buffalo from the Kruger National Park were screened. The LAMP assay that targets the PIM and p150 genes of *T. parva* was used as an additional independent test. The conventional, SYBR Green and LAMP PCRs correlated well with each other as well as the real-time PCR for the majority of negative and *T. parva* positive samples. Some positive samples were missed with the protein genes, possibly due to sequence variation in the primer regions. The protein genes did, however, detect additional positive samples diagnosed as negative by the real-time PCR. These samples were all *T.* sp. (buffalo) positive and indicate possible suppression of PCR signal due to template competition because of mixed infections. Independent markers can thus be useful for accurate diagnosis of *T. parva* infection where mixed infections occur in buffalo.

The malarial drug targets, DXR and GCHI: co-expression with molecular chaperones and homology modelling

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Malaria, caused by *Plasmodium* strains, is the world's most important parasitic disease, causing at least 1 million deaths annually. Drug-resistant parasite strains have developed, and research on putative malarial drug targets is frequently hampered by low yields of soluble protein obtained by heterologous expression in *Escherichia coli* host cells. Different strategies were examined to improve the production of folded, functional target protein. The approaches were to co-express the target protein with molecular chaperones, optimisation or harmonisation of the coding region and co-expression where the conditions of transcription and induction were highly controlled. The molecular chaperone *Pf*Hsp70 was used as it has been shown to facilitate the correct folding and assembly of proteins in the parasite cell. *Plasmodium falciparum* 1-deoxy-*D*-xylulose 5-phospate reductoisomerase (*Pf*DXR) catalyses fatty acid biosynthesis, using a pathway different from the human melvalonate pathway. Co-expression with the molecular chaperone *Pf*Hsp70 enhanced over-expression of *Pf*DXR from a codon-harmonised coding region. There were substantial improvements in the yield of *Pf*DXR following control of transcription of the *Pfdxr* gene. The expression problem was overcome for the drug target GTP cyclohydrolase I (*Pf*GCHI), from the malarial folate biosynthetic pathway, by co-expression of *Plasmodial* molecular chaperone *Pf*Hsp70. The *Pf*GCHI was successfully purified in milligram quantities and its identity confirmed by mass spectroscopy. This is the 1st time that malarial GCHI has been produced and purified in quantities suitable for structural and functional studies. Both *Pf*DXR and *Pf*GCHI have been shown to have *in vitro* activity in enzyme assays. These co-expression systems should be applicable to other malarial proteins recalcitrant to soluble expression in heterologous systems. Homology modelling studies for these drug target proteins have been undertaken. A 3-dimensional homology model for *Pf*DXR and the C-terminal region of *Pf*GCHI have been created and validated using structure-checking programmes. The *Pf*DXR model was used to develop an efficient approach to screen for potential tool compounds *in silico* for use in the rational design of novel inhibitors. After sufficient optimisation of purification, large yields of folded, functional target proteins will be used for screening potential inhibitors and structure determination. This may then assist in the development of novel, clinically effective antimalarial drugs.

Loop-mediated isothermal amplification (LAMP) assays for specific detection of *Trypanosoma vivax* infections in livestock and tsetse flies

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Trypanosoma vivax trypanosomosis is widespread in sub-Saharan Africa and in Central and South America and in Africa is transmitted by tsetse flies but other blood-feeding insects such as horse-flies and stable-flies are capable of mechanical transmission. Loop-mediated isothermal amplification (LAMP) is a rapid DNA amplification technique that amplifies DNA with high sensitivity and specificity under isothermal conditions. In the current study we have developed 2 specific LAMP assays targeting the cathepsin-L-like and diagnostic antigen genes with detection limits of 100 fg and 10 pg, respectively, for serially diluted *T. vivax* DNA. DNA extracted from cattle samples collected in Zambia and Tanzania and DNA extracted from tsetse flies captured in Zambia were used for detection of *T. vivax* infections by the Cathepsin-L LAMP assay and the cathepsin-L PCR assay in order to compare the 2 assays. Five out of 200 (2.5 %) cattle DNA samples and 2/36 (5.5 %) goat DNA samples from Zambia were positive for the presence of *T. vivax* infections by both cathepsin LAMP and PCR assays. Furthermore, both cathepsin-L LAMP and cathepsin-L PCR assays similarly detected 12/260 (4.6 %) of *T. vivax* infections from tsetse DNA samples collected in Zambia. These results indicate the potential of LAMP to detect *T. vivax* infections in both the host and the vector.

Anthelmintic resistance in small ruminants in the emerging farming sector in the Gauteng Province, South Africa

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Resistance to anthelmintics in livestock has reached high levels on commercial farms in South Africa, and its occurrence in the resource poor farming sector was also reported in 1999. It was, therefore, the aim of the current study to determine anthelmintic resistance in sheep and goats in the emerging farmers' sector in Gauteng Province. Faecal Egg Count Reduction Test (FECRT) was used to determine anthelminthic resistance (AR) in small stock and prior to experiments a questionnaire was administered to each participating farmer to obtain information about their herd structure and previous use of anthelminthic drugs. Three experiments were conducted on separate farms, with 2 experiments on sheep and the 3rd experiment on goats. Three groups of anthelminthic drugs, namely macrocyclic lactones (ML), levamisole (Lev) and benzimidasole (BZ) were screened. Faecal cultures were prepared both pre- and post-treatment to identify the occurrence of resistant nematodes. FECRT results for the treatment of sheep on the 2 farms were as follows: ML (98 %; 90 %), Lev (95 %; 98 %) and BZ (99 %; 73 %). The results of the treatment of the goats on a 3rd farm were as follows: ML (93 %), Lev (94 %) and BZ (66 %). *Haemonchus* sp. was identified as the dominant resistant nematode on the 3 farms. The results showed that AR (FECR % ≤95 %) was present in both the ML and BZ groups in sheep and in all 3 anthelminthic groups in goats. Since AR was detected in all 3 groups of drugs, corrective measures should be applied, particularly for the BZ group, as the FECR % was very low (66 and 73 %). The use of the ML group should, however, be monitored. The detected AR in this study can be attributed to drug mismanagement and this calls for more animal health extension programmes by Government that include AR awareness and training in the correct use of anthelmintic drugs.

A symbiotic copepod collected from the blue coral worm *Pomatoleios kraussii* (Polychaeta)

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During surveys conducted along the South African coastline, a variety of intertidal invertebrates and tidal pool fishes were collected and examined for the presence of symbionts. Amongst these, the blue coral worm *Pomatoleios kraussii,* was found to be infested with a parasitic copepod. Small colonies of worms were collected from the rocky shores. Individual worms were separated and examined with a dissecting microscope. The copepods were found attached between the radioles near the branchial crown and specimens were removed and fixed in 70 % ethanol for further analysis. To date no males have been collected. Of the 350 worms examined, 140 (42 %) were infested with 1, sometimes more, with a maximum of 5 female copepods. A variety of copepod families are associated with marine invertebrates: Pseudanthessidae usually associate with echinoderms, but a few species are known from polychaetes. The bodies of these copepods are often modified or totally transformed. Clausiidae are more commonly found on polychaetes. These copepods have a cyclopiform to elongated body, segmented appendages and reduced legs, with the terminal segment excentrically implanted. All the representatives of the Herpyllobiidae (except *Herpyllobius hartmanae*) are found to parasitise polynoid polychaetes. The body of these copepods has 2 unsegmented parts, the embedded endosoma and the protruding ectosoma bearing the genital apertures. The adult females have no appendages. The morphology of the blue coral worm copepod differs from all known species and is probably new to science.

Poster Presentations

The characterisation of *Babesia* spp. in felids in southern Africa

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Babesia is an intracellular erythrocytic haemoprotozoan of mammals but has also been reported in reptiles and birds. The 2 most frequently reported *Babesia* species in felids are *B. felis,* which causes clinical babesiosis in domestic cats, and *B. leo,* primarily reported from asymptomatic lions. In this study DNA was extracted from blood collected from various domestic, captive and free-ranging felids from different southern African countries. The V4 hypervariable region of the 18S rRNA gene was amplified and the PCR products were analysed using the Reverse Line Blot (RLB) hybridisation assay. RLB probes to detect *B. felis, B. leo* and *B. lengau* were used to screen samples collected from various felid species. Results showed that *B. felis* and *B. leo* occurred more frequently in the host from which they had initially been described, namely domestic cats and lions respectively, but were also detected in other felid species. A large number of samples reacted only with the *Babesia/Theileria* genus-specific probe. These samples were further analysed using 18S rRNA gene sequence- and phylogenetic analysis. Phylogenetic studies confirmed the presence of *B. lengau* in cheetahs and *Hepatozoon* in lions. It also suggested the existence of a novel *Babesia* species in domestic cats.

Studies on *Haemogregarina fitzsimonsi* Dias, 1953, a blood protozoan, as a possible bio-indicator for measuring South African wild tortoise population health

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South Africa has the world's richest biodiversity of terrestrial tortoises. A number of these species make popular pets, but their exportation/importation may lead to global transmission of reptilian parasites/infections. Heartwater, a bacterial infection carried by the African tortoise tick, *Amblyomma marmoreum,* and transmissible to ruminants, is 1 such example. However, there is little research

concerning apicomplexan haematozoans of chelonians, or any estimation of their relationship with tortoise health. Recent studies on South Africa's tortoise haematozoans, the 1st such observations since those of Laveran in 1905, have recorded 4 species of apicomplexans from 7/14 species of tortoises. Interestingly, 1 of these 4 parasite species, *Haemogregarina fitzsimonsi* Dias, 1953, appears to be a generalist, infecting all 7 tortoise species across a range of eco-regions. First described in Mozambique and thought to be transmitted by ticks, *H. fitzsimonsi* displays increased prevalence and parasitaemia in captive and likely stressed hosts in South Africa, hosts on which vectors may not be properly controlled. However, in wild conditions parasite prevalence is much more variable, and parasitaemia is low. It is hypothesised that *H. fitzsimonsi* may be a useful bio-indicator for wild tortoise population health, since it appears relatively well adapted to its wild hosts. As the life cycle of this parasite still requires elucidation, host blood continues to be examined and ticks collected for study. Tortoise body condition (body mass/shell volume) appears to correlate with parasitaemia. On average, with increased host body condition, prevalence of infection and parasitaemia are reduced.

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

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The African buffalo is known to be the reservoir host of numerous important tick-borne pathogens, most of which are carried asymptomatically, but which can cause disease if transmitted to susceptible animals. Of these, *Theileria parva,* the causative agent of East Coast fever, Corridor disease (a controlled disease in South Africa) and January disease, and *Ehrlichia ruminantium,* the causative agent of heartwater, are considered to be the most important tick-borne disease agents of livestock in sub-Saharan Africa. No mortalities due to heartwater have yet been documented in the African buffalo, but it is known that a subclinical carrier state can occur in buffalo. This suggests that buffalo could play an important role in the epidemiology and spread of heartwater and therefore could serve as reservoirs of infection that may represent a threat to the livestock industry. As little is known about the *Ehrlichia* spp. infection status of African buffalo, the primary objective of this study was to determine the occurrence of *Ehrlichia* species in buffalo samples collected from 2 game parks in South Africa. These samples were simultaneously screened for the presence of *Anaplasma, Theileria* and *Babesia* spp. DNA was extracted from 200 buffalo blood samples originating from the Kruger National Park and the HluhluweiMfolozi Park (KwaZulu-Natal province), South Africa and subjected to the Reverse Line Blot (RLB) hybridisation assay. *Ehrlichia* sp. Omatjenne, *Anaplasma marginale* and *A. centrale* were detected in 70 % of the samples. The presence of these parasites has not been previously reported in South African buffalo populations. None of the samples tested positive for *E. ruminantium.* Of the piroplasm parasites included in the assay, *T. parva, Theileria* sp. (buffalo), *T. mutans, T. buffeli* and *Babesia occultans* were identified. The detection of *B. occultans* is of interest since this is mostly a cattle parasite that has not been reported before in buffalo. The PCR products of 5 samples only hybridised with the genus-specific probe, suggesting the presence of novel species and/or variants of species in the buffalo population and will be further investigated by cloning and sequencing of the PCR products.

The historical site of Sir David Bruce at Ubombo

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The site, consisting of structural and artefactual remains believed to be those of the research station where Dr David Bruce (later Sir) and his wife Mary worked between 1894 and 1897, have recently been discovered at the small village of Ubombo in Northern KwaZulu-Natal (the former Zululand). It was at this locality that Dr Bruce discovered the causative agent of nagana (*Trypanosoma brucei brucei*). He furthermore established the role of the tsetse fly (*Glossina pallidipes*) as vector in the transmission of nagana between game and domestic animals. The site fits the meagre, albeit significant, description and information presented by Dr Bruce in his writings on the location of this specific site. This justified an archaeological investigation to test the hypothesis that it is indeed the place where Bruce lived and where he conducted his research. The findings will further form a basis for the reconstruction of the house and research station and also investigations into the roles played by various people during the specific period.

Marine fish parasitic isopods (Cymothoidae) from the southwestern Indian Ocean, including records of 2 new species

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Cymothoid isopods are a unique parasitic group rarely studied in Africa, and thus information on their biodiversity, occurrence, distribution and hosts is scanty. These data are necessary to understand the effects these parasites have on their host populations and specifically the effects on hosts targeted as potential mariculture species. Within the family Cymothoidae is a group, commonly known as tongue-replacement isopods or tongue-biters, found within the buccal cavity. A recent review of *Cymothoa,* 1 of the tongue-biter genera, shows approximately 48 species worldwide, of which only 3 are from the southwestern Indian Ocean. Owing to the naturally high biodiversity of this region, this low number is most likely not a true reflection of the actual species numbers present. To test this hypothesis, cymothoid isopods were collected during the past 2 years from Zanzibar, Tanzania, as well as from the east coast of South Africa. Additional material from the South African Museum in Cape Town was also obtained to clarify their taxonomic status. The isopod collected in Zanzibar was removed from the buccal cavity of the parrotfish, *Leptoscarus vaigiensis*, and after comparison with known species, was found to be new to science. Likewise, a new species was discovered from Sodwana Bay, South Africa, in the mouth of the large-spotted pompano (*Trachinotus botla*) with a prevalence of 73 %. This work increases the number of *Cymothoa* species in this region to 5, which is expected to increase further with more sampling and revision of the other Cymothoidae genera in southern Africa.

Observations on the pathology of ergasilids from Lake Tanganyika on the gills of *Lamprichthys tanganicanus*

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Four species of *Ergasilus* have been described from fishes in Lake Tanganyika. During a recent survey specimens of *Ergasilus sarsi* were found on the gills of *Lamprichthys tanganicanus* at Lubumba in the vicinity of Baraka. Specimens and gills were collected after the fish were killed by severing the spinal cord. Parasites were fixed intact on the gills in an acetoformaldehyde alcohol solution and preserved in 70 % ethanol prior to dehydration and embedding in resin. Serial sections were made at 5 μm and stained with a trichrome stain with azocarmine and azan solution. Superficial tissue erosion was observed in the vicinity of the 2nd antennae, maxillipeds, and even swimming legs. Fusion of the secondary lamellae occurred due to epithelium hyperplasia. Mucous cell proliferation was observed at the interface between host and parasite and Rodlet cells were observed. Ruptured blood vessels were present and blood cells and

gill tissue were observed in the intestine of the parasite. The prevalence of the parasite was 100 % and the number of parasites per host extremely high, so that fish respiration could be impaired and reduced feeding, weight loss, and general deterioration of health can result. Gill functioning is additionally impaired by the reduced oxygen-binding ability of water at higher temperatures such as that encountered in lakes in tropical Africa.

The difference between natural and cultured conditions – a case study of metazoan parasites of Mozambique tilapia

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Seasonal parasitological surveys were conducted from June 2009 to April 2010 at the Flag Boshielo Dam (FBD) as part of a larger project on the health of *Oreochromis mossambicus* (Mozambique tilapia). FBD forms part of the Olifants River System. During the study, fish of the same species in grow-out earth ponds at the Aquaculture Unit (AU) of the Tompi Seleka Agricultural College near FBD showed signs of stress and reduced growth. This initiated parasitological surveys at the AU and comparison between cultured and natural fish populations. All endo- and ectoparasites were fixed and preserved using conventional methods. The following parasites were recorded from both conditions (abundance indicated): *Cichlidogyrus* spp. (3.83 at FBD and 0.84 at AU); *Clinostomum* sp. (0.03 at FBD and 1.06 at AU); digenean larvae from gills (0.10 at FBD and 0.19 at AU); *Ergasilus* sp. (0.10 at FBD and 0.77 at AU). Gryporhynchid larvae (0.23), *Contracaecum* larvae (0.23), and digenean larvae from the skin (0.03) were recorded only at FBD. *Gyrodactylus* sp. (0.03) and *Euclinostomum* sp. (0.06) were recorded only at AU. The digenean metacercariae were more abundant under cultured conditions, while the monogeneans were more abundant under natural conditions. These ponds at AU are frequently visited by piscivorous birds that feed on the fingerlings, essential for completion of the digenean parasitic life-cycle. In addition to the considerable damage that the digenean metacercariae cause to the musculature and viscera of fish, they make the fish unattractive to consumers. Parasitic infection and fish disease are significant causes of economic loss in the aquaculture industry.

Possible presence of cattle-derived *Theileria parva* parasites in South Africa

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Cattle- and buffalo-derived *Theileria parva* parasites cause East Coast fever (ECF) and Corridor disease, respectively, in cattle. ECF was eradicated in southern Africa in 1955 but Corridor disease still occurs in South Africa and is a controlled disease. Following seasonal outbreaks of what was initially suspected to be babesiosis on a farm near Ladysmith, KwaZulu-Natal, South Africa, the presence of *T. parva* was confirmed on this farm. In this study, *T. parva* parasites originating from this farm were characterised by sequence analysis of the variable regions of the genes coding for the antigens p67, p104 and the polymorphic immunodominant molecule (PIM).

DNA samples isolated from blood obtained from 37 cattle from the farm were screened for *T. parva* DNA using the *T. parva* real-time PCR assay. The variable regions of the p67, p104 and PIM genes were amplified from 14 *T. parva*–positive samples. Amplicons produced from 6 samples were cloned into a plasmid vector before sequencing. Cattle-type p67, p104 and PIM alleles were obtained from samples and the inferred amino acid sequences were similar to that of *T. parva* Muguga, a cattle stock responsible for ECF in Kenya. Buffalo-type (p67 and PIM) and novel (p104) alleles were obtained from the remaining 4 samples. For the 1st time, mixed-type PIM sequences, composed partly of cattle-type sequences and partly of buffalo-type sequences, were identified. Findings from this study indicate that *T. parva* parasites with cattle-type p67, p104 and PIM alleles were present on a farm near Ladysmith in South Africa, suggesting the possible presence of cattle-derived parasites. However, ECF was not diagnosed in these animals and ECF has not been reported in South Africa since its eradication in the 1950s. Identification of novel p104 and mixed-type PIM sequences suggests extensive genetic diversity in the *T. parva* population in South Africa.

Blood parasites of African rhinoceroses and their impact on conservation

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Trypanosomosis and babesiosis have been implicated in mortalities in both black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceroses in East and southern Africa. Subclinical carrier states have recently been demonstrated by molecular techniques. There is mounting evidence that endemic stability, which may exist for both diseases, can be disrupted by stress, *e.g.* capture and translocation. Furthermore, rhinoceroses growing up in areas where specific vectors are rare or absent (*e.g.* ticks in arid areas) may be fully susceptible if infected later in life. Rhinoceroses occur in geographically dispersed populations. Metapopulation management, which implies translocation of individuals between populations to maintain genetic diversity, may result in individuals succumbing to either of the 2 diseases. Controlled exposure and chemoprophylaxis are possible preventative measures.

Variability in infection indices and diversity of metazoan parasites of *Schilbe intermedius* from 2 dams in the Limpopo Province of South Africa – can water quality play a role?

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Schilbe intermedius was sampled at 2 localities in Limpopo Province, South Africa. Fifty specimens from Nandoni Dam in former Venda and 56 specimens from Flag Boshielo Dam near Marble Hall were examined for metazoan parasites. Fish were collected during different seasons of 2009 and 2010 by using gill nets. All endo- and ectoparasites were fixed using standard methods and preserved in 70 % ethanol. The parasitological data were compared with water quality data from the 2 localities. *Schilbetrema quadricornis* (Monogenea), *Contracaecum* sp. larva (Nematoda), *Paracamallanus cyathopharynx* (Nematoda) and *Clinostomum* sp. metacercaria (Trematoda) were recorded from both localities. *Dolops ranarum* (Branchiura), *Diplostomum* sp. metacercaria (Trematoda) and an unidentified larva (Trematoda) were recorded only from Nandoni Dam. Hosts from Nandoni Dam had a greater diversity and higher prevalence of parasites than those from Flag Boshielo Dam. This could be due to the slightly poorer water quality at Flag Boshielo Dam compared to Nandoni Dam. The water quality may have a direct effect on the parasites themselves or on the prevalence of their intermediate or final hosts, preventing the life cycle of the parasites from being completed. The prevalence and intensity of ectoparasites was also lower at Flag Boshielo Dam. This may be because they are directly in contact with the poorer quality water. Fish parasites may be used as indicators of water quality because of the variety of ways in which they respond to the ecosystem they inhabit.

Sequence heterogeneity in the ribosomal DNA internal transcribed spacer among *Theileria* spp. from water buffalo and cattle in China

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Theileria is a widespread, intraerythrocytic, tick-borne protozoan parasite that causes mild to severe infections in its hosts. In order to define the molecular epidemiology of the *Theileria* in China, 34 *Theileria* isolates were obtained from water buffalo and cattle from different geographical locations in China, the ribosomal DNA internal transcribed spacer and the 5.8S rRNA gene (ITS1-5.8S rRNA-ITS2) were amplified by PCR, then cloned and sequenced. Four types (~1000 bp, ~1200 bp, ~1500 bp and ~2400 bp) of the ITS1-5.8S rRNA-ITS2 gene were obtained. Phylogenetic analysis of these gene sequences revealed that 11 000 bp sequence belonged to *Theileria annulata,* 191 200 bp sequences, 52 400 bp sequences belonged to the *Theileria sergenti/buffeli/orientalis* group and 101 500 bp sequences belonged to unidentified *Theileria* spp and possibly a novel *Theileria.* The results showed that each *Theileria* species possessed specific nucleotide sequences, this also suggested that the *Theileria* species in China could be distinguished by the PCR product size of the ITS1-5.8S rRNA-ITS2 gene.