Parasitological Society of Southern Africa

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Keynote Addresses

Climatic change over South Africa: Projections and perceptions

F Engelbrecht

Department of Geography, Geoinformatics and Meteorology, University of Pretoria, Pretoria, 0002 South Africa. E-mail: francois.engelbrecht@up.ac.za

Most scientists agree that the warming Planet Earth has experienced over the last 4 decades can be ascribed to anthropogenic forcing – specifically the burning of fossil fuels and deforestation. The gradual melting of the polar ice caps, the retreating glaciers of the Northern Hemisphere and reduced snowfall over Marion Island and Mount Kilimanjaro are all thought to be fingerprints of Global Warming and Climate Change. But how will the climate of South Africa be affected by this global problem? In particular, how will rainfall patterns over South Africa change over coming decades? This presentation will explain how mathematical models of the atmosphere, *i.e.* Global Circulation Models (GCMs), can be used to project how climate will change in response to enhanced greenhouse gas concentrations. Emphasis will be placed on the uncertainties that are associated with these projections on future climate. From this background, it will be shown that there is insufficient proof for the perception that 'South Africa will become wetter in the east and drier in the west' as a result of Global Warming. In fact, it is plausible that the country will become generally drier. It will be shown that there is strong evidence to suggest that the winter rainfall region of South Africa will become significantly drier in the future climate. Eastern South Africa may be expected to experience shorter summer rainfall seasons with more intense rainfall events, although this region may also be expected to become drier on the average. Finally perspective will be given on the perception that Climate Change can still be prevented. It will be shown that the world is already committed to inevitable anthropogenically induced climate change.

Is *Plasmodium knowlesi* the 5th human malaria parasite?

R H Hunt and M Coetzee

University of the Witwatersrand and the National Institute for Communicable Diseases, Johannesburg, South Africa. E-mail: maureenc@nicd.ac.za

Plasmodium knowlesi is a monkey malaria 1st described in the 1930s from southeast Asia. Early laboratory experiments showed that this parasite would infect and develop in humans, but there are few recorded naturally occurring infections that remained sporadic and the zoonotic potential limited. The effects of infection with *P. knowlesi* are usually mild and not considered to be life-threatening. However, when used for 'malaria therapy' of syphilis to treat general paralysis of the insane, the passage from human to human produced an increasingly virulent strain of parasites and *P. knowlesi* was abandoned in favour of the milder *P. vivax*. The morphology of *P. knowlesi* closely resembles *P. malariae* and is frequently misdiagnosed as this malaria. Recent work carried out in Malaysia, using PCR to identify the parasite, showed that human infection with *P. knowlesi* is common and widespread and, furthermore, was fatal in 4 patients. The rapid replication of *P. knowlesi* every 24 h means that prompt diagnosis and treatment is essential to prevent hyper-parasitaemia and associated organ failure. The emergence of a virulent strain with properties similar to *P. falciparum* is cause for concern and has lead to speculation that *P. knowlesi* is somehow being transmitted from human to human, although mosquito transmission is sufficient to account for the widespread dissemination of the disease. There is a real possibility that carriers of *P. knowlesi* may travel to Africa and research is needed to determine whether our common vectors here can transmit this parasite too.

Oral Presentations

Development of a quantitative real-time PCR assay for the detection of *Babesia caballi* infections in equids

R Bhoora[1], M Quan[1,2], A J Guthrie[2] and N E Collins[1]

[1]*Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa. E-mail: raksha.bhoora@up.ac.za*

[2]*Equine Research Centre, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa*

A quantitative TaqMan® minor groove binder (MGB) real-time PCR assay was developed for the detection of *Babesia caballi* infection in equids from South Africa. Sequences of the V4 hypervariable region of the 18S rRNA gene, obtained from 16 *B. caballi*-positive field samples, were used to develop the assay. The detection limit of the assay was determined to be 53 parasites/mℓ of blood. The assay was tested on samples collected from 41 horses, resident on 3 poorly managed stud farms. The presence of piroplasm DNA in these samples was determined using the reverse-line blot hybridisation assay (RLB). RLB results showed that 30 samples were positive for *Theileria equi,* 2 samples hybridised to only the *Theileria/Babesia* genus-specific probe and 9 samples were negative. Nineteen of the samples that were positive for *T. equi* also hybridised to the *Babesia* genus-specific probe but not the *B. caballi* species-specific probe. These 19 samples were, however, all positive for *B. caballi* DNA by real-time PCR assay. The assay also detected *B. caballi* DNA in 7 of the remaining 11 samples that were positive only for *T. equi* on RLB. Furthermore, the real-time PCR assay was able to detect *B. caballi* DNA in 1 of the 2 samples that hybridised only to the *Theileria/Babesia* genus-specific probe as well as in 1 of the 9 samples that were tested negative on RLB. The Taqman® MGB™ real-time PCR assay thus proved to be more sensitive than the RLB assay in the detection of positive *B. caballi* infections.

Identification of *Theileria mutans* genotypes from African buffalo using 18S rRNA gene sequence analysis.

M Chaisi[1,2], F Potgieter[3], K Sibeko[1], M Troskie[1], N Collins[1] and M Oosthuizen[1]

[1]*Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa. E-mail: s27130712@tuks.co.za*

[2]*Department of Biology, National University of Lesotho, Roma 180, Lesotho*

[3]*Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa*

The African buffalo (*Syncerus caffer*) is the natural reservoir host of pathogenic and non-pathogenic *Theileria* species. Corridor disease, caused by *Theileria parva,* is a controlled disease in South Africa. Buffalo are the natural reservoir host of *T. parva,* as well as 2 other *Theileria* species infecting cattle, the relatively benign *T. mutans* and the apathogenic *T. velifera*. *Theileria buffeli* and the hitherto uncharacterised *Theileria* sp. (buffalo) have thus far only been identified in some buffalo populations in South Africa. *Theileria* parasites usually occur as mixed infections and although the benign and non-pathogenic forms do not have any significant economic importance, they can interfere with the diagnosis of the pathogenic forms. Recently, buffalo blood samples originating from the Kruger National Park and the Hluhluwe-Imfolozi Park were screened for *Theileria* species using the Reverse Line Blot (RLB) hybridisation assay. Results demonstrated the presence of *T. parva, T. mutans, T. velifera, T. buffeli* and *Theileria* (sp.) buffalo, either as single infections or mixed infections. In a number of samples the PCR products did not hybridise with any of the *Babesia* or *Theileria* species-specific probes, only with the *Babesia/Theileria* genus-specific probe, suggesting the presence of a novel species or variant of a species. Full-length 18S rDNA of 2 samples were amplified, cloned and sequenced.

Phylogenetic analyses indicated that a *Theileria* species infection was present showing highest similarity with *T. mutans.* Species-specific RLB oligonucleotide probes were designed and will be used to screen buffalo samples to determine the prevalence of these genotypes in buffalo in South Africa.

Abalone tubercle mycosis an emerging disease in the South African abalone industry

K Christison[1], B Macey[1] and A Mouton[2]
[1]*Department of Environmental Affairs and Tourism, Marine and Coastal Management, Private Bag X2, Roggebaai, 8012 South Africa. E-mail: kchris@deta.gov.za*
[2]*Amanzi Biosecurity, Private Bag X15, Suite 190, Hermanus, 7200 South Africa*

An abalone tubercle mycosis has recently been discovered in abalone culture facilities in South Africa. Infected abalone are characterised by multifocal areas of necrosis of the epithelium, underlying muscle fibres and connective tissues of the foot, epipodium and mantle. The lesions are typically 2–3 mm in diameter and consist of an epithelial defect, sometimes covered in loosely adherent off-white material, surrounded by a thin black reaction zone. In advanced cases, the lesions enlarge and may coalesce to affect large areas of tissue. Diseased animals are weak and show a tendency to sit at the top of baskets and may even climb out of the water. Affected aquaculture facilities have suffered significant production losses, with up to 90 % mortality in spat and up to 30 % mortality in older animals. Pure cultures of the fungus have been isolated from tissue samples of infected abalone. We have demonstrated that mycelium, grown in liquid media, produces copious suspensions of spores within 24 h of being washed and immersed in sterile sea water. Zoospores are highly motile and comprise 2 flagella. The morphology of the fungal mycelium and zoospores is consistent with members of the Class Oomcyetes. Molecular characterisation of the fungus, the pathogenicity and the possible relationship with environmental conditions is currently being investigated.

The velvet assassins – *Amyloodinium ocellatum* potential disease risk for captive marine fishes in South Africa

K W Christison[1] and D B Vaughan[2]
[1]*Department of Environmental Affairs and Tourism, Marine and Coastal Management, Private Bag X2, Roggebaai, 8012 South Africa. E-mail: kchris@deta.gov.za*
[2]*Two Oceans Aquarium, PO Box 50603, Victoria & Alfred Waterfront, Cape Town, 8000 South Africa*

Amyloodinium ocellatum is regarded as a devastating prolific ectoparasitic dinoflagellate of fish in high densities or in confinement. This parasite has a direct life-cycle and under optimal conditions parasite loads increase rapidly culminating in clinical disease and death. Traditionally this geographically widespread pathogen is considered to occur only in warm temperate and tropical regions. Following an outbreak of this pathogen at the quarantine facility at the Two Oceans Aquarium, tomonts were recovered from infected fish and incubated at various temperatures (15, 18, 20, 22, 24 and 26 °C). Temperature had a marked effect on the rate of dinospore production with optimal dinospore production occurring around 24 °C. Marine aquaculture finfish candidate species, dusky kob (*Argyrosomus japonicus*), were challenged and found to be susceptible to this pathogen. Parasites were recovered from the skin and gills. Histopathological investigation revealed a significant inflammatory response accompanied by severe hypertrophy of the gill epithelia culminating in fusion of the secondary gill lamellae. Little associated pathology was observed on the skin and fins of infected fish. The extreme virulence exhibited by *Amyloodinium ocellatum* during this study combined with the rapid increase in parasite intensity presents a significant risk for fish in captivity in public aquaria as well as in the developing marine finfish culture industry all around our coastline but particularly as interest in finfish mariculture gains more interest in the Eastern Cape and KwaZulu-Natal.

The ultrastructure and possible function of the spiracle in ticks

F Clarke[1], E D Green[2] and C Baker[3]
[1]*Department of Biology,* [2]*Department of Anatomy,* [3]*Electron Microscope Unit, University of Limpopo (Medunsa Campus), Box 139, MEDUNSA, 0204 South Africa. E-mail: clarke@ul.ac.za*

It is generally accepted that the spiracles in ticks control both respiratory gas exchange and tracheal water loss. The ultrastructure of the spiracles of *Amblyomma marmoreum,* an exceptionally large tick, was investigated through scanning electron microscopy, to try and determine how these functions are performed. Flat and engorged adults were fixed live in 70 % ethanol, while others were 1st euthanased in CO_2 before fixation. Spiracles were dissected and processed for scanning electron microscopy (SEM) and viewed in a Leica Stereoscan 420 SEM at 5–7 kV. The spiracular plate comprises a comma-shaped outer margin perforated by many aeropyles, surrounding a macula with a central ostium, plugged by a valve-like lip. The interpedicellar space consists of a labyrinth of pedicels connecting the spiracular plate to the floor of the subostial space. The subostial space leads into the atrial chamber from which the main tracheal trunks arise. A valve-like mechanism may close off the atrial chamber and trachea from the subostial space, and may thus limit tracheal water loss through the open aeropyles when the ostium is closed. The dense arrangement of pedicles and the closely spaced small aeropyles may further increase the resistance to transpiration and reduce the airflow, thereby reducing the water vapour loss from the spiracles. Ducts perforate the base plate, connecting the interpedicular space with the body cavity. Condensation droplets forming on the pedicels could drain back into the haemolymph, thus rehydrating the tick daily, when dew point is reached.

Blood dietary factors that may influence the survival and production in tsetse flies colonies

C J de Beer, G Venter and F Potgieter
Parasites, Vectors and Vector-borne Diseases (PVVD), ARC-Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort, 0110 South Africa. E-mail: debeerc@arc.agric.za

Mass rearing of tsetse flies is dependent on the sustainable availability of a high quality blood diet. In any mass rearing facility, the logistics for obtaining sterile, high quality fresh blood from abattoirs are challenging. An added complication is the influence of potential chemical, physical and microbiological contaminants in the blood of donors as well as contamination during the collection, handling and storage. Research at the ARC-OVI has been directed towards the development of quality assurance procedures for the supply of the *in vitro* diet for the maintenance of productive colonies of *Glossina brevipalpis* and *Glossina austeni.* The effects of anticoagulants and the suitability of bovine, porcine and bovine/porcine blood mixtures as a diet were investigated. The influence of bovine growth hormones as blood contaminants on tsetse development was investigated. A 25-day bioassay (Feldmann 1994) was used to determine the effects of these factors on tsetse productivity. Out of the 5 anticoagulants evaluated, Citrate Phosphate Dextrose Adenine (CPDA) and Acid Citrate Dextrose (ACD) gave the best results. EDTA was found to be toxic to the flies. Diet mixtures of 50 % bovine/porcine blood were found to be best suited for *G. brevipalpis* and a mixture of 75 % bovine blood and 25 % porcine blood for *G. austeni.* Bovine growth hormones, widely used in South Africa in feedlots, can be expected to be present in the blood obtained from commercial abattoirs. Comparisons of blood collected from negative controls and cattle herds treated with growth hormones demonstrated no significance difference in tsetse fly productivity. Indications are that blood dietary factors may have an important effect on the mass production of tsetse flies.

Metal accumulation analysis within tissue of *Bothriocephalus acheilognathi*

N Degger and A Avenant-Oldewage
Department of Zoology, Auckland Park Kingsway Campus, University of Johannesburg, PO Box 524, Auckland Park, 2006 South Africa. E-mail: aoldewage@uj.ac.za

The use of parasites as bioindicators has increased rapidly since

the 1980s. The majority of parasite-pollution indicator studies have shown that parasites, particularly acanthocephalans and cestodes, accumulate metals at higher concentrations than their hosts. The results of these studies have, however, shown a disparity in metal concentrations between their anterior and posterior sections. Thus the objective of this study was to separate the cestode *Bothriocephalus acheilognathi* into head, mature and gravid segments and analyse their individual metal accumulation capacity by ICP-OES and ICP-MS for 23 elements. In an attempt to implement metal ion detection on a visual basis, the scope of the study expanded into fluorochromy. The results of the mean metal concentration comparisons showed that many of the elements analysed in this study were found in higher concentrations in the cestode (specifically in the head tissue) when compared with its host with exception to Ti, V, Ba and Sr, which were found to be in higher concentrations in the host spinal cord. The bioconcentration factor ($BF = C_{\text{parasite tissue}}/C_{\text{host tissue}}$) results showed that out of the 23 elements analysed, *B.acheilognathi* head tissue bioconcentrated 9, mature tissue 6 and gravid tissue 5 metals, revealing a high variation in accumulation trends. The fluorochromy results were promising, indicating that visual detection of metal ions is possible within cestode tissue. In conclusion, the results have shown that *B. acheilognathi* is a macroconcentrator, an organism with uneven metal accumulation throughout its body, a pattern which may be due to differences in calcareous corpuscle distribution.

Effect of deworming on EPI programmes

C Dochez[1], S Dube[2], A Meheus[1] and A Hoosen[3]
[1]*Network for Education and Support in Immunisation (NESI), Department of Epidemiology and Social Medicine, University of Antwerpen, Universiteitsplein 1, 2610 Antwerpen, Belgium. E-mail: carine.dochez@ua.ac.be*
[2]*Department of Microbiological Pathology, University of Limpopo, Medunsa Campus, South Africa*
[3]*Department of Medical Microbiology, University of Pretoria, PO Box 2034, Pretoria, 0001 South Africa*

Worldwide, the burden of disease associated with helminth infections (mainly schistosomiasis and soil-transmitted helminths) is high. The global incidence rate amounts to about 2 billion cases, of which a significant proportion involve children. Chronic infections have a negative impact on the general health, the cognitive development, the physical fitness and the iron status of those infected, and can also affect their immunological status. Helminthic infections have a similar geographic distribution pattern to a number of infectious diseases, like HIV infection, TB and malaria. In South Africa, the national burden of helminthic disease is unknown, but local studies have shown a high prevalence of intestinal nematode infections in the provinces of Western Cape, Mpumalanga, and KwaZulu-Natal. Although TB vaccine coverage is high in South Africa, there are still more than 100 000 new TB cases annually, and 20 % of those occur in children under the age of 5. South Africa is currently also experiencing one of the most severe HIV/AIDS epidemics in the world. Recent research suggests that helminthic infections alter the immune status of individuals, possibly leading to a reduced effectivity of the vaccine. Helminth infections lead to a pronounced T-helper type 2 (TH2) immune profile. Persons with a pre-existing dominant TH2 profile may not be able to generate a TH1 type response. Where a high load of helminth infection exists, prevention of major epidemic diseases by vaccination could therefore be less effective. A high priority needs to be given to studying the interactions between worms and other infectious agents, in order to define the optimal conditions for both the deworming and immunisation programmes.

Parasites and diseases of amphibians: Should we be concerned about Madagascar?

L du Preez
School of Environmental Sciences and Development, Potchefstroom Campus, North-West University, Private Bag X6001, Potchefstroom, 2520 South Africa. E-mail: louis.dupreez@nwu.ac.za

Like all other vertebrate classes, amphibians too are plagued by a variety of pathogens. These includes bacterial infections, viral infections, fungal infections, and parasitic infections including a wide variety of protozoans, trypanosomes, monogenetic flukes, digenetic flukes, tapeworms, acanthocephalans, nematodes and pathogenic arthropods such as dipteran larvae and mites. Amphibians do have a well-developed and effective immune defence system and the majority of pathogens will not cause serious illnesses. The clawed frog *Xenopus laevis* for example serves as host for no less than 25 different parasite genera representative of all major parasite groups except the acanthocephalans and they appear to cope very well with these multiple infections. Amphibians, however, do get infected by 2 extremely lethal pathogens, namely a group of closely related viruses grouped in the genus *Ranavirus* and the amphibian chytrid fungus *Batrachochytrium dendrobatidis*. Both are classified as emerging infectious diseases and were placed on the Wildlife Diseases List by the OIE (Office International des Epizooties), the World Organisation for Animal Health. We have seen in recent years how chytrid infections swept through large geographical areas killing off frogs in their thousands. Frogs on several continents have suffered sudden high mortality rates, resulting in various species becoming extinct and many others undergoing massive population reductions. The global decline in populations of amphibians is one of the most vexing conservation issues of recent times. A recent report from the IUCN's Global Amphibian Assessment suggests that as many as a 3rd of amphibian species have undergone severe declines or extinction with over 7 % listed as critically endangered and many species on the brink of extinction. A question that comes to mind is but what about Madagascar? With its unique and diverse anuran fauna an outbreak of either amphibian chytrid or *Ranavirus* could undoubtedly have catastrophic consequences. The time to investigate and take action is now!

Study of an ectoparasitic crustacean from Malaysia with light and electronmicroscopy

L Everts and A Avenant-Oldewage
Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park, Johannesburg, 2006 South Africa. E-mail: aoldewage@uj.ac.za

Seven specimens of an unknown freshwater ectoparasitic crustacean were collected from red tilapia fish kept for consumption at the 'Langat Fishing, Seafood and Beer Garden' Restaurant just off the Langat River in Malaysia. Initial investigation showed that the specimens were of the genus *Argulus*. Light and scanning electron microscopy (SEM) studies were subsequently used to identify the species. A comparison with all *Argulus* species previously described from Asia and the surrounding islands was conducted. The species was identified as *Argulus coregoni* Thorell, 1866 due to the presence of the roughly triangular shaped anterior respiratory areas and the kidney bean shaped posterior respiratory areas. Additionally, the abdomen with sharply pointed terminal ends as well as the presence of characteristic accessory protrusions on the 2nd swimming leg of the male specimens confirmed this identification. The accessory protrusion structures consist of 1 small and 1 large protrusion on each leg, rounded terminally and covered by scales. This species has not previously been described from Malaysia.

The functional microstructure of the cabbage alphid *Brevicoryne brassicae* (L.)

E D Green
Department of Anatomy, University of Limpopo (Medunsa Campus), Box 232, Medunsa, 0204 South Africa. E-mail: edward@ul.ac.za

These plant parasites are important pests attacking members of the Cruciferae. They are not only phytophagous phloem feeders, but also may transmit a number of economically important plant viruses. This study investigated the microstructure of these parasites to determine how they are specialised to attach to, feed and reproduce on their host plants, using scanning electron microscopy (SEM). Parts of the infested leaves were collected and fixed in 70 % ethanol. The insects were then cleaned by ultrasonication before being routinely prepared for SEM. Gold-plated specimens were viewed at various angles in a Leica 420 stereoscan at 5–8 kV. The sucking mouthparts are comprised of protusible mandibular and maxillary stylets lying in a grooved labium which formed the sap-feeding rostrum. A number of sensilla were observed on the

mouthparts which would be important in probing and penetrating the leaf. Although the antennae were long, relatively few sensilla were present. The lateral compound eyes were composed of numerous rounded ommatidia but certain ommatidia were enlarged and elongated extending out laterally as the ocular tubercles. The long legs ended in 2-jointed, 2-clawed tarsi which seemed to close laterally. A pair of tubular cornicles which are abdominal tubes through which they are reported to exude droplets of honeydew, alarm pheromones or a quick-hardening defensive fluid called cornicle wax were present. The posterior tip of the abdomen bore a tail-like protrusion called a cauda above their rectal apertures. Fine-toothed setae and sensory sensilla covered the surface of the cauda. These micromorphological specialisations may also be useful in future comparative taxonomic studies of aphids.

The biodiversity, systematics and ecology of fish parasitic gnathiid isopods from the East Coast of South Africa

K A Hadfield, N J Smit and A Avenant-Oldewage

Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park, 2006 South Africa. E-mail: kerryh26@yahoo.com

In South Africa, gnathiid isopod larvae have been recorded parasitising various intertidal fish hosts along the West and South Coasts. However, the warmer East Coast has an intertidal fish species composition which differs dramatically from the other 2 coasts. To establish whether this is also true for gnathiids, 4 trips, 1 in each season, were undertaken in 2006 and 2007 to collect intertidal fishes and their parasitic gnathiids from the East Coast of South Africa. Gnathiids that completed their feeding on fish hosts were kept alive in order to elucidate the life cycle and to study the feeding ecology of this isopod. A total of 316 fishes belonging to 17 species were collected of which 15 were parasitised by gnathiids. The preferred hosts for this new species were identified as *Scartella emarginata, Antennablennis bifilum* and *Istiblennius dussumieri* with a prevalence of 89.6 %, 77.2 % and 74.5 %, respectively. The descriptions for the male, female and praniza larva were done using light and scanning electron microscopy following standard techniques, and the average life cycle, from the 1st larval stage to adult, took between 134 to 140 days. Aspects of the ecology of this new gnathiid species were also analysed to determine host preference, gnathiid prevalence and intensity, as well as the possible correlation between the host size and gnathiid abundance using univariate (CANOCO) and multivariate (SPSS) statistical tests. *Scartella emarginata* was found to have the highest prevalence, intensity and abundance and a positive correlation was also seen between host size and gnathiid abundance.

Infections and mortalities induced by *Metarhizium anisopliae* in various developmental stages of the red-legged tick *Rhipicephalus evertsi evertsi* using 2 formulations

M Hedimbi[1], G P Kaaya[2] and P M Chimwamurombe[1]

[1]*Department of Biological sciences and* [2]*Faculty of Agriculture, University of Namibia, Private Bag 13301, Windhoek, Namibia.*
E-mail: gkaaya@unam.na/mskaaya@hotmail.com

The objective of this study was to investigate the pathogenicity and hence the biocontrol potential of *Metarhizium anisopliae* to *Rhipicephalus evertsi evertsi,* an economically important tick of livestock in Africa that transmit *Babesia equi* to horses, *Anaplasma marginale* to cattle and cause paralysis in lambs, adult sheep and calves. Infection of eggs and larvae was achieved by placing conidial suspensions on filter papers in 65 mm plastic disposable Petri dishes and placing 40–50 tick eggs or larvae on them, after which they were incubated at 26 °C and 90 % relative humidity (Rh). Nymphs and adults were infected by dipping them for 5 seconds in various conidial concentrations, placed in 65 mm disposable Petri dishes and incubated at 26 °C and 90 % Rh. Fungal infections and mortalities in eggs and larvae were detected using a dissecting microscope for a period of 21 days post-exposure to the fungal conidia. Mortalities in nymphs and adults were also recorded daily for 21 days. The eggs were successfully infected by *M. anisopliae* when exposed to conidia of this entomopathogen both in water and oil formulations. The proportion of infected eggs increased with increasing conidial concentration and was higher in oil than in water formulation. Likewise, mortalities in larvae, nymphs and adults increased with increasing conidial concentrations and were also higher in oil than in water formulations. There was no difference in mortality between unengorged and engorged adult ticks exposed to the same conidial concentrations. Our results indicate that *M. anisopliae* is highly pathogenic to all developmental stages of the red-legged tick *R. e. evertsi* and therefore warrants further investigation for its potential as a biocontrol agent for this tick species.

Comparative features of *Argas walkerae* and *Argas persicus* using scanning electron- and light microscopy

H Heyne and J Putterill

ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, South Africa 0110. E-mail: heyneh@arc.agric.za

In the early 1970s, previous collections of *Argas persicus* in South Africa were renamed *Argas walkerae* with the presumption that *A. persicus,* as a separate entity, was not present in South Africa. During a tick survey (2001–2003) conducted in the North West Province of South Africa, a relatively large number of tampans collected in the Kudumane area off poultry and in a kraal were identified as *Argas persicus sensu stricto.* Two specimens collected off poultry from another farm, also in the Kudumane area, were identified as *A. walkerae.* The main morphological feature used in comparing and identifying the 2 species is the different shape of the peripheral (marginal) cells. Scanning electron- and stereo microscopy were used to illustrate this feature in adult specimens.

An egg-laying rhythm in *Diplectanum oliveri* (Monogenea: Diplectanidae), a gill parasite of dusky kob *(Argyrosomus japonicus)*

C J H Joubert[1], K W Christison[1] and H Kaiser[2]

[1]*Department of Biodiversity and Conservation Biology, University of the Western Cape, Private Bag X17, Bellville, 7535 South Africa.*
E-mail: caspervet@telkomsa.net
[2]*Department of Ichthyology and Fisheries Science, Rhodes University, Grahamstown, 6139 South Africa*

Globally the production of fish in aquaculture enterprises is suffering from serious infections with monogeneans of which 4 genera have been identified from *Argyrosomus japonicus* (dusky kob) and *Argyrosomus inodorus* (silver kob) in local mariculture facilities. Of these monogeneans, *Diplectanum oliveri* has been recorded in the highest prevalence and intensities in both wild caught fish stocks as well as in farmed stocks. Recent innovative approaches to quantifying monogenean infections on fish, by counting the eggs produced by infra-populations of these parasites over a 24-hour period, may be a reliable method to quantify the intensity of an individual infrapopulation of parasites on a single host. An egg-laying rhythm in *D.oliveri,* a gill parasite of dusky kob was investigated *in situ* with 9 infected fish under a controlled lighting schedule of 12-hour illumination and darkness cycle starting at 06:00 with the illumination phase. Individual fish were kept in nine, 20-litre, well-aerated containers at 20 °C. Eggs were collected from individual fish every 4 hours once the trial commenced, by decanting the total volume of water through a 25-μ mesh. Each sample was washed into plastic jars and preserved. A total of 8763 eggs was collected from 54 samples over 24 hours. The lowest egg production was during illumination phase 13:00–17:00 with an average of 116.1 and the highest during darkness phase 01:00–05:00 with an average of 229.9 which demonstrates a well-defined egg-laying rhythm

Control of the major malaria vector *Anopheles gambiae* displaying resistance to multiple insecticides in Ghana

M Kaiser[1,3*], R H Hunt[1,2], L L Koekemoer[1,3], B D Brooke[1,3] and M Coetzee[1,3]

[1]*Vector Control Reference Unit, National Institute for Communicable Diseases, NHLS, Private Bag X4, Sandringham, 2131 South Africa.*
E-mail: oliverw@nicd.ac.za
[2]*School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag X3, University of the Witwatersrand, Johannesburg, South Africa*

[3]*Division of Virology and Communicable Disease Surveillance, School of Pathology of the National Health Laboratory Service and the University of he Witwatersrand, Johannesburg, South Africa*

Malaria is holoendemic in Ghana and is responsible for 22 % of mortality in children under 5 as well as 9 % of maternal deaths. The main malaria vectors in Ghana are *Anopheles gambiae* and *An. funestus.* In 2004 the AngloGold/Ashanti goldmine in Obuasi implemented a vector control programme using indoor residual spraying (IRS). Following the success of this programme the Newmont goldmine at Ahafo also adopted a malaria control programme. An initial survey was conducted to determine the insecticide susceptibility of the malaria vectors at the mine and in surrounding villages to the 4 classes of insecticides currently approved for IRS. These are organochlorines, organophosphates, carbamates and pyrethroids. *Anopheles gambiae* were exposed to all 4 classes using standard WHO bioassays and were found to be resistant to 3 of these classes. In a subsequent survey the mosquitoes displayed resistance to all 4 classes. This poses a very serious threat to the control of malaria vectors in the region. To successfully reduce malaria transmission in the area a strict integrated control programme using IRS, insecticide treated bed nets, and methods to limit suitable breeding sites of the vectors needs to be implemented. Communities surrounding the mine must be involved in order to effectively control and reduce the economic burden of malaria.

Is the *Culicoides* fauna changing? Port Elizabeth as a case study

K Labuschagne and G J Venter

Parasites, Vectors and Vector-borne Diseases (PVVD), ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa. E-mail: labuschagnek@arc.agric.za

Culicoides (Diptera: Ceratopogonidae) biting midges transmit a number of important pathogens to livestock. In South Africa, African horse sickness (AHS) and bluetongue (BT) viruses are the 2 most important viruses transmitted by these midges. *Culicoides imicola* was considered to be the only vector of AHS and BT prior to 1998. It is the most abundant species collected near livestock and more than 1.4 million can be collected in a single light trap. In the Port Elizabeth area of the Eastern Cape Province *C. imicola* is rarely collected while *C. bolitinos* is the most abundant species collected. Port Elizabeth was thus considered to be safe from AHS outbreaks as *C. imicola* was absent in the area. This changed when AHS virus was isolated from *C. bolitinos* during an outbreak of AHS in the Clarens area of the Free State Province. An intensive survey conducted from 1995–1999 on Ascot stud in Port Elizabeth confirmed the rarity of *C. imicola* on this farm. Only 5 specimens of *C. imicola* were collected in the 170 collections made. An additional survey was conducted from 2006 on the same farm. To date 53 collections were made and 446 specimens of *C. imicola* collected. The monthly averages for total *Culicoides*, *C. imicola* and *C. bolitinos* during both surveys indicate changes in abundance and seasonal distribution. Possible causes for these changes include management, hygiene and environmental changes on the farm.

Seasonal distribution of *Culicoides* spp. on 2 farms in the Western Cape Province

K Labuschagne and G J Venter

Parasites, Vectors and Vector-borne Diseases (PVVD), ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa. E-mail: labuschagnek@arc.agric.za

Culicoides (Diptera: Ceratopogonidae) biting midges transmit a number of important pathogens to livestock. In South Africa, African horse sickness (AHS) and bluetongue (BT) viruses are the 2 most important viruses transmitted by these midges. To facilitate the export of horses a quarantine station and AHS free zone was established in the Western Cape Province. During 1999 and 2004 the Western Cape Province experienced 2 outbreaks of AHS with devastating consequences each time the zone was closed and a ban placed on export of horses. Owing to these outbreaks funding was obtained to do an intensive survey of the Stellenbosch area of the Western Cape. *Culicoides* collections were made utilising 220 V Onderstepoort-type light traps. The seasonal distribution of *Culicoides* species collected on 2 farms during the 1st year of survey is discussed. The 2 farms chosen as having the most complete data sets for 2006 were Wilgerbosdrift, north of Piketberg and Broadlands stud near Gordons Bay. The total monthly *Culicoides* averages for all the species as well as for *C. imicola* and *C. bolitinos* were compared with each other as well as separately. *Culicoides* abundances and vector-free periods were investigated.

Epidemiology of animal trypanosomosis in northern KwaZulu-Natal 2005–2007

A Latif[1], L Ntantiso[2], P Majiwa[1] and P Van Den Bossche[3]

[1]*Parasites, Vectors and Vector-borne Diseases (PVVD), ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa. E-mail: latifa@arc.agric.za*
[2]*Josine State Veterinary Office, South Africa*
[3]*Faculty of Veterinary Science, UP& ITVM, Antwerp, Belgium*

Two species of tsetse flies exist in northern KwaZulu-Natal, namely *Glossina brevipalpis* and *G austeni,* occupying about 16 000 km^2. We report here, as part of the epidemiological studies of the disease carried out from 2005, the association between infection in cattle with trypanosomes and ensuing anaemia. Three communal dip tanks, Ekuphindisweni (Eku), Mvutshini (Mvu) and Ocilwane (Oci) neighbouring the edge of Hluhluwe Game Reserve were selected for the study. H-tsetse traps were installed at each of the 3 locations and the traps were visited for fly collection once every 2 weeks. Blood in EDTA was collected from cattle on each location at a monthly interval and examined by detecting mobile trypanosome parasites using the haematocrit concentration technique (buffy coat examination). The packed cell volume (PCV) for each animal was recorded. Tsetse fly catches were high in the 2 locations, Eku and Mvu, and very low at Oci, which lies at the most southern limit of the fly distribution. Subsequently, 50 %, 60 % and 100 % of the infected cattle with trypanosomes in Eku, Mvu and Oci dip tanks, respectively, had anaemia (PCV of 24 % or less). Anaemia in uninfected cattle in the 2 locations under the high tsetse challenge was 20 % and 30 % compared to 10 % of cattle with anaemia in the low tsetse challenge. Overall, 62 % of the trypanosomes infected cattle were anaemic while only 20 % of the uninfected cattle had anaemia. Cattle at the 3 locations were examined for presence of internal parasites and the results showed that only 2/90 cattle had high faecal egg counts of 1500/g. In conclusion, the study demonstrated a clear association between trypanosomes infections in cattle and anaemia.

Aspects of the ecology of *Cichlidogyrus philander* collected in the Padda Dam from *Pseudocrenilabrus philander philander*

L le Roux and A Avenant-Oldewage

Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park, 2006 South Africa. E-mail: aoldewage@uj.ac.za

Monogeneans of the genus *Cichlidogyrus* parasitise the gills of the Cichlidae. Generally monogeneans are host-specific, in this genus both oioxenic and stenoxenic specificity occurs. During an ecological study 245 fishes were collected with the aid of hand nets, from the Padda Dam, during monthly surveys. Fish were transported to the laboratory and killed by severing the spinal cord. Gills were inspected with the aid of a dissection microscope. The parasite specimens were fixed and mounted in a glycerine-ammonium-picrate solution. Their distribution over the gill arches was noted and the data processed. Only *Pseudocrenilabrus philander philander* was parasitised by *Cichlidogyrus philander* indicating a strict host specificity. The number of specimens varied from 1 to 184 per fish, at a prevalence of 93 %, with a mean intensity of 18.3 and a mean abundance of 17.7. It was concluded that it has no preference for host sex. Prevalence was correlated to the total length of the host. It was determined that the number of parasites does not influence the condition factor of the fish. No significant difference between the number of parasites collected from gill arches on the left or right sides of fish, nor a preference for a specific gill arch was recorded. The preferred sites of attachment of the parasites were the distal ends of the gill filaments. It was determined that there is no correla-

tion between season and the prevalence. This is the 1st record of this species in South Africa and the 1st ecological study conducted on it.

Infection of *Anopheles* species with the rodent malaria parasite *Plasmodium berghei.*

T M Lo[1,2], L L Koekemoer[1,2] and M Coetzee[1,2]

[1]*Vector Control Reference Unit, National Institute for Communicable Diseases, National Health Laboratory Services (NHLS), Private Bag X4, Sandringham, 2131 South Africa. E-mail: markl@nicd.ac.za*

[2]*Division of Virology and Communicable Disease Surveillance, School of Pathology of the National Health Laboratory Service and the University of Witwatersrand, Johannesburg, South Africa.*

Malaria remains one of the most deadly human infectious diseases and is responsible for over a million deaths a year in Africa. Mosquitoes belonging to the genus *Anopheles* are vectors of the *Plasmodium* parasites, the causative agent for malaria. Recent research has shown that the mosquito immune system responds to the presence of the parasites, although very little is known about these interactions. A good model for looking at these interactions is the rodent malaria *P. berghei*. Lab-bred mice strains (C57/Black and Balb/c) were infected with *P. berghei* parasites. 0.2 – 0.3 mℓ of infected blood was injected into 6–8-week-old mice interperitoneally. Four to 5 days post-infection; adult female mosquitoes were allowed to feed on the anaesthetised infected mice. Fourteen to 18 days after the initial blood meal, the female mosquitoes' salivary glands were dissected out and viewed under light microscopy. Sporozoites, 10–20 μ in length, were found to congregate around the ruptured infected salivary glands. G3 and SUA strains of *Anopheles gambiae* have been found to be viable vectors of *P. berghei* thus far. This experience will be applied to experimental infections of *Anopheles funestus*, another major malaria vector. Colonies with confirmed sporozoite presence can be used as vectors to transmit the parasite between mice. With the establishment of a complete parasite lifecycle between the host and vector, the parasites will remain infective and cause appropriate host immune responses which can be studied.

Parasites – a cost of sociality?

H Lutermann and N C Bennett

Mammal Research Institute, Department of Zoology & Entomology, University of Pretoria, Pretoria, 0002 South Africa. E-mail: hlutermann@zoology.up.ac.za

Parasitism is often assumed to be a cost of sociality and individuals living in larger groups are usually expected to exhibit higher rates of infection with parasites. However, evidence in support of this proposition is equivocal and it has been suggested that in group living organisms lower between-group transmission rates may compensate for higher within-group transmission rates. We evaluated the latter hypothesis in the social Natal mole-rat (*Cryptomys hottentotus natalensis*) that exhibits reproductive division of labour and breeds throughout the year. Animals were collected during bimonthly captures from March 2003 to January 2004 in Glengarry Park in the Kamberg region and infection with the cestode *Raillietina* sp. was assessed during dissections of the individuals captured. For a total of 230 individuals collected from 59 different colonies the prevalence of infection with *Raillietina* sp. was 32.6 %. The observed prevalence was not affected by sex, reproductive status or capture month. Furthermore, for complete colonies we found a non-linear relationship between group size and parasite prevalence with groups of intermediate size being more likely to be infected with *Raillietina* sp. Group identity was the single most important factor predicting parasite prevalence for Natal mole-rats with usually either all or no individual in a group being infected. Our results suggest that high group stability and strong social barriers to inter-group dispersal could reduce the spread of parasites in a population.

The 1st adult gryporhynchid cestode from freshwater fish

W J Luus-Powell[1] and S N Mashego[2]

[1]*Department of Biodiversity, University of Limpopo (Turfloop Campus), Private Bag X1106, Sovenga, 0727 South Africa. E-mail: powellw@ul.ac.za*

[2]*North-West University, Mafikeng Campus, Private Bag X6001, Potchefstroom, 2520 South Africa*

Adult gryporhynchid cestodes occur commonly in piscivorous birds such as herons, spoonbills and cormorants and freshwater fishes serve as intermediate hosts for the larvae (metacestodes). During parasitological field surveys of freshwater fishes, adult cestodes were found in the intestine of mormyrid hosts which superficially resembled the proteocephalid cestodes. However, these cestodes have more characteristics of the Cyclophyllidea and could be assigned to the family Gryporhynchidae. Specimens were fixed with hot alcohol-formalin-acetic acid (AFA) and stored in 70 % ethanol. Some cestodes were stained in Horen's Trichrome and scoleces of 2 specimens were mounted in Berlese's fluid for measurements of hooks. Standard methods were followed for scanning electron microscopy and to study the histomorphology. The scolex is quadrilobulate, each lobe bearing a prominent sucker. The scolex bears a terminal rostellum with 1 crown of alternating large and slightly smaller hooks. The rostellum is retractable within a large, spindle-shaped muscular rostellar pouch. The rostellar hooks are numerous. A distinct, unsegmented neck is present. The complete strobila is small. The immature proglottids are acraspedote. Muscle bundles are well developed. The testes are very numerous and postovarian, located entirely in the posterior end of the proglottid in 1 continuous field. The ovary is small and bilobed. The cirrus is unarmed and the cirrus pouch is swollen posteriorly to accommodate an internal vas deferens. The vitellarium is compact, situated between the 2 lobes of the ovary. The uterus changes during maturation, disappearing with eggs loose in parenchyma. The ovary, testes, vitelaria and uterus are all situated in the medulla.

Molecular detection of trypanosomes infecting cattle, wild animals and tsetse flies in KwaZulu-Natal, South Africa

M V Mamabolo, L Ntantiso, A Latif and P A O Majiwa

ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa. E-mail: mamabolomv@arc.agric.za

Limited information exists regarding the prevalence and incidence of trypanosomosis in South Africa, particularly in KwaZulu-Natal (KZN) since the 1990 outbreak. There have also not been systematic attempts to describe the species of trypanosome in KZN with molecular tools. The aim of this study was to use molecular biological reagents (primers targeting different genomic loci) and methods (polymerase chain reaction (PCR) combined with RFLP) to detect and characterise trypanosomes in cattle, wild animals and tsetse flies in KwaZulu-Natal. The analyses were conducted on samples collected from cattle at 14 dip tanks and 1 commercial farm around the Hluhluwe-Imfolozi Game Reserve, wild animals within Hluhluwe-Imfolozi Game Reserve and tsetse flies from a commercial farm, Hluhluwe-Imfolozi Game Reserve and 2 other Game Reserves. A total of 673 cattle, 266 tsetse flies, 141 buffaloes and 6 rhinoceros samples were analysed. The studies revealed the following: at least 2 species of trypanosomes exist in KZN: *T. congolense* and *T. vivax.* The 2 species occur as single and as mixed infections in cattle. Moreover, the *T. congolense* in the infections were found to comprise 2 genotypic groups: the Savannah-type and the Kilifi-type, which were present either as single or mixed infections in cattle and in tsetse flies. Maximum infection rates observed were 92 % in cattle, 4.3 % in buffaloes and 97.5 % in tsetse flies. We also observed that cattle nearer to game reserves had higher infection rates than those located further away.

Risk analysis of potential transmission and implications of exotic *Gyrodactylus* spp. on cultured and wild cyprinids in the Western Cape

M R Maseng[1], K Christison[1,3] and C Griffiths[2]

[1]*Department of Biodiversity and Conservation Biology, University of the Western Cape, Private Bag X17, Bellville, 7535 South Africa. E-mail: 2319394@uwc.ac.za*

[2]*Department of Zoology, University of Cape Town, Private Bag X3, Rondebosch, 7701 South Africa*

[3]*Department of Environmental Affairs and Tourism, Marine and Coastal Management, Private Bag X2, Roggebaai, 8012 South Africa*

Koi and goldfish are well-known vectors of foreign parasites globally, some of which have infected local indigenous fish. These fish have also been implicated in the global transfer of the notorious genus *Gyrodactylus.* Representatives of the genus *Gyrodactylus* are

among the most invasive of fish parasites and their single direct life-cycle, viviparity, and polyembryonism contribute greatly to their invasive success. The study aims to identify *Gyrodactylus* spp. present on introduced koi carp and goldfish, as well on wild local cyprinids. *Gyrodactylus* on the body surfaces of the fish, either purchased commercially, or wild caught samples, were removed, quantified and morphologically identified. Measurements of the haptoral organs, which included a total of 20 point to point measurements of the hamuli, ventral bar, dorsal bar and marginal hooklets were made. *Gyrodactylus kherulensis* Ergens, 1974, and *G. kobayashii* Hukuda 1940, were identified from koi carp and goldfish, respectively, and a previously undescribed species of *Gyrodactylus* was found on the body surfaces of *Pseudobarbus burchelli*, a local endemic cyprinid. Mass importations of *G. kherulensis* and *G. kobayashii* from China, Japan and Malaysia are currently taking place and they have been recorded at prevalences ranging from 0–100 % and mean intensities of 2–342.3. *Gyrodactylus* species play an integral role in aquaculture health management generally by compromising both the integrity and economics of the aquaculture industry. These parasites also pose a major potential threat to the biodiversity and health status of local cyprinids in the Western Cape.

Implication of trypanosome strains diversity in the epidemiology of bovine trypanosomosis

J Masumu[1] and P Van Den Bossche[1,2]

[1]*Department of Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa. E-mail: jmasumu@hotmail.com*
[2]*Institute of Tropical Medicine, Animal Health Department, Nationalestraat 155, B-2000 Antwerp, Belgium*

The expression of bovine trypanosomosis undergoes considerable variations over space and time throughout the infested area in sub-Saharan Africa. To determine the implication of the parasite in this process, 37 *Trypanosoma congolense* Savannah isolates were collected from cattle in eastern Zambia and characterised for (1) genetic profiles using a modified Amplified Fragments Length Polymorphism (AFLP) technique, (2) virulence in mice, (3) transmissibility using *Glossina morsitans morsitans* and (4) cross-protection between isolates using the 'infection-challenge' method. The results obtained revealed a high genetic variability among these isolates. Different genotypes were found to circulate in different areas. The virulence characterisation showed a considerable variation among these strains with some strains being highly virulent whereas others (the majority in this endemic area) were of moderate or low virulence profile. An uneven distribution of these strains was also observed, with highly virulent strains being prevalent in some areas whereas the majority of strains in other areas were of either moderate or low virulence profile. There was also considerable variation in the transmissibility of these strains, with highly virulent strains being easily transmitted by *G. m. morsitans* compared to strains of moderate or low virulence profile. When mice were infected with low virulent strains and subsequently challenged with highly virulent strains, variation was also observed in the outcome of the infection after challenge. In some cases, animals were protected against the adverse effects of highly virulent strains while in other cases animals were not protected. The outcome of this study suggests that the characteristics of trypanosome strains circulating in cattle in a given area and the interaction between them may play an important role in the expression of the disease.

Monogenean parasites of largemouth bass *Micropterus salmoides* (Lacepede, 1802) in Tzaneen Dam

M M Matla[1], N M Mokgalong[1] and S N Mashego[2]

[1]*Department of Biodiversity, University of Limpopo (Turfloop Campus), Private Bag X1106, Sovenga, 0727 South Africa. E-mail: matlam@ul.ac.za*
[2]*North-West University , Private Bag X2046, Mmabatho, 2735 South Africa*

This forms part of a larger project on the helminth parasites of freshwater fish in Tzaneen Dam (23°47'40"S, 30°09'40"E). *Micropterus salmoides* is an alien fish species and was introduced into the dam through active stocking programmes. As a popular game fish species many sponsored bass fishing tournaments are organised at the dam. Monogenean parasites have been found on this fish species in the USA as well as in other countries where it was introduced, but no records of any monogeneans on this fish exist from Africa. The main focus was to examine the fish specimens for any monogenean parasites and identify them through morphological analyses. Host species (n = 32) were caught using gill nets as well as hook and line and were examined for monogeneans on the skin, gills and in the ureter-urinary bladder complex. Those procured were fixed in 70 % alcohol and mounted on slides with the aid of glycerine jelly dissolved over a flame. Morphological analyses were done using a BMX 50 Nomarski clinical microscope fitted with a drawing tube, a digital camera and an eyepiece with a μm calibrated measuring ruler. The results revealed a prevalence of 60 % for monogenean infections. Three species of monogeneans were found; *Acolpenteron ureterocoetes* (n = 3) from the ureter-urinary bladder; *Actinocleidus fusiformis* (n = 432) and *Haplocleidus furcatus* (n = 12) co-occur on the gills where they appear as macroscopically similar. Differential diagnoses of the gill monogeneans revealed *A. fusiformis* with 2 pairs of anchors approximately of uniform size and shape and *H. furcatus* with 2 pairs of anchors similar in shape but markedly dissimilar in size. A complete morphological analysis was done for each of the 3 monogeneans to confirm their status as new as well as the 1st records on *M. salmoides* in Africa.

Aspects of the biology and biogeography of chelonian polystomes

C Morrison, L H du Preez and C Weldon

School of Environmental Sciences and Development, Potchefstroom Campus, North-West University, Private Bag X6001, Potchefstroom, 2520 South Africa. E-mail: christa.morrison@student.nwu.ac.za

Monogeneans are hermaphroditic flatworms that are primarily external parasites of fish. One family, the Polystomatidae, radiated onto the tetrapods and are mainly found in amphibians and aquatic chelonians. This study focused on the polystome genera *Neopolystoma, Polystomoides,* and *Polystomoidella* that parasitise terrapins. Although their general morphology is fairly uniform, they differ in the number of anchor hooks (hamuli) located on the haptor. *Neopolystoma* has no hamuli, while *Polystomoidella* has 1 pair, and *Polystomoides* has 2 pairs. Because polystomes need water to transmit to new hosts, only aquatic chelonians can function as hosts. Furthermore, they occur in sites on the host that are accessible from the exterior, such as the eye cavity, mouth, nasal cavity, pharynx, oesophagus, intestine, cloaca, and the urinary bladder.

Two of these genera, *Neopolystoma* and *Polystomoides,* have cosmopolitan distributions, while *Polystomoidella* has a very limited range. The geographical distribution of the species can be linked to anthropogenic influence in terms of host translocation, and as a reflection of research effort on chelonian polystomes. Despite this, genetic evidence has found that both the parasites, as well as their chelonian hosts, have diversified 200 million years ago, proving that the parasites are also bound to their hosts' natural distribution, as well as being more related to each other than to other polystome genera. Host diversity and distribution also explain why certain polystome species have a more dispersed distribution. Present studies have identified 2 new *Neopolystoma* species from the eye cavity of 2 terrapin species from the Gainesville area in Florida, USA.

Vector competence of field and colony *Glossina austeni* and *Glossina brevipalpis* for trypanosome species in KwaZulu-Natal

M Y Motloang[1], J Masumu[2], P Van Den Bossche[2,3], P A O Majiwa[1] and A A Latif[1]

[1]*Parasites, Vectors and Vector-borne Diseases (PVVD), ARC-Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort, 0110 South Africa. E-mail: motloangm@arc.agric.za*
[2]*Department of Veterinary Tropical Diseases, University of Pretoria, Onderstepoort, 0110 South Africa*
[3]*Institute of Tropical Medicine, Veterinary Department, Nationalestraat 155, 2000 Antwerpen, Belgium*

Tsetse-transmitted trypanosomosis constitutes a major constraint to livestock and agricultural production in Africa affecting 37 countries including South Africa. The presence of *Glossina austeni* and

G. brevipalpis in northern KwaZulu-Natal represents the southernmost limit of distribution of the tsetse fly belt. In the present study, the vectorial competence of *G. austeni* and *G. brevipalpis* in transmitting pathogenic trypanosomes in cattle was assessed. A total of 468 *G. brevipalpis* and 43 *G. austeni* were collected in KwaZulu-Natal from July 2006 to January 2007. The collections included 7 batches of *G. brevipalpis* which were fed on 7 susceptible bovines, 1 batch/bovine, and 3 batches of *G. austeni* fed on 2 bovines and 1 goat (1 batch/animal). All animals were kept in the insect-free facility at the Onderstepoort Veterinary Institute (OVI), monitored every 2nd day for the presence of trypanosomes in buffy coat smears, daily for the development of anaemia and body temperature. In all animals challenged with wild *G. austeni* (two bovine and 1 goat) parasitaemia was detected from day 7 to 22 post-infection while no parasites could be detected in any of the animals (7 bovines) challenged with *G. brevipalpis.* Subsequently, parasitaemic animals ($n = 3$) were used to feed 900 teneral tsetse flies of each species reared at OVI insectory (300 flies were used per infected animal). Infection rates with trypanosomes were assessed in these flies. Twelve per cent of proboscis infections (mature) and 19 % of midgut infection only (immature) were found in *G. austeni.* Only 4 % of immature infections were seen in *G. brevipalpis.* It is concluded that *G. austeni* is the primary vector of trypanosomes in KwaZulu-Natal.

Monitoring performance in South African parasitology proficiency testing programmes, 2003–2007

B Poonsamy, L Dini and J Frean

Parasitology Reference Unit, National Institute for Communicable Diseases, 1 Modderfontein Road, Sandringham, South Africa. E.mail: bhavanip@nicd.ac.za

Clinical laboratories in South Africa recognise the importance of quality assurance systems. Such systems aim to ensure that laboratories provide accurate and reliable results. Quality assurance systems usually include participation in external quality assessment (EQA) programmes (proficiency testing schemes). We examined the performance of participants in our parasitology EQA schemes over a 5-year period. The results of all diagnostic laboratories that participated in 2 of our EQA programme (blood parasites and stool parasites) from 2003 through 2007 were analysed. These are mostly South African laboratories (both public and private), as well as some laboratories from other African countries. We calculated the percentage acceptable results (scores of ≥75 %) for all 16 surveys of each programme, and analysed them to identify any possible trends. No clear trend in the percentage acceptable results over the years was found, for either EQA parasitology programmes. We found that 13 surveys of the stool programme and 11 surveys of the blood programme had percentage acceptable results that fell between 35 % and 65 %. The average percentage acceptable results over the 5 year period were 50 % for the stool programme and 54 % for the blood programme. Most laboratories, despite presumably giving their best effort, performed poorly in the EQA programmes. There are plausible reasons for the overall poor EQA performance. These can serve as the source of the solution on how management can improve both EQA and routine patient results. Participants need to be encouraged to use the valuable tools provided by EQA to their benefit.

Development of a TaqMan PCR for the detection of *Theileria parva* in South Africa

N E Rambritch, A A Latif, B Mans, R Pienaar, E Zweygarth, A Josemans and F T Potgieter

Parasites, Vectors and Vector-borne Diseases (PVVD), ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa. E-mail: paplin@arc.agric.za

Theileria parva (*T. parva*), the cause of Corridor disease, is transmitted from carrier buffalo to cattle by the hard tick, *Rhipicephalus appendiculatus.* In South Africa this is a controlled disease affecting the cattle farming industry therefore it is necessary by law that before buffalo are to be translocated to non endemic areas they have to be certified free of Corridor disease. The ARC-OVI is mandated to test for *T. parva* in South Africa. The current molecular diagnostic test is the real-time hybridisation probe PCR. Research results suggest that the test amplifies a closely related *T. sp.* (buffalo). An improved real-time TaqMan PCR based on the 18s rRNA gene was developed and is currently being validated. DNA was extracted from serial dilutions of the blood from an experimental *T. parva* carrier animal with 0.02 % parasitaemia. DNA from other *Theileria* species, blood parasites and bacteria were subjected to the TaqMan PCR to determine the analytical specificity. The analytical sensitivity of the TaqMan assay was found to be 0.00002 % parasitaemia with 100 % certainty, which is equivalent to 3.5 parasites/ 2.5 $\mu\ell$. The results thus far show that the TaqMan assay does not amplify any other *Theileria* species, related haemoparasites, Gram-positive or Gram-negative bacteria. The TaqMan PCR assay appears to be a rapid, sensitive and specific molecular test for *T. parva* diagnosis.

The anthelmintic efficacy of copper oxide wire particles (COWP) against *Haemonchus contortus* in communally grazed indigenous goats in KwaZulu-Natal, South Africa

A Spickett[1], J F de Villiers[2], J D F Boomker[3], J B Githiori[4], G F Medley[5], M O Stenson[1], P J Waller[6] and A F Vatta[1]

[1]*Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa. E-mail: spicketta@arc.agric.za*
[2]*KwaZulu-Natal Department of Agriculture and Environmental Affairs, Private Bag X6005, Hilton, 3245 South Africa*
[3]*University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.*
[4]*International Livestock Research Institute (ILRI), PO Box 30709, 00100, Nairobi, Kenya*
[5]*University of Warwick, Department of Biological Sciences, Coventry CV4 7AL, United Kingdom*
[6]*Department of Parasitology (SWEPAR), National Veterinary Institute (SVA), SE-751 89, Uppsala, Sweden (deceased)*

Haemonchus contortus is considered to be the most economically important gastrointestinal nematode of small ruminants in the tropical and subtropical areas of the world. Control of the parasite is complicated, however, by a high prevalence of anthelmintic resistance worldwide. As an alternative to conventional anthelmintics, copper oxide wire particles (COWP) offer considerable promise, but their anthelmintic efficacy under field conditions needs to be comprehensively assessed. To this end, their efficacy was examined in goats raised under communal farming conditions in the Bergville area, KwaZulu-Natal Province, South Africa. The faecal nematode egg counts of 187 female indigenous goats belonging to 15 farmers (2–35 animals per farmer) were monitored at 4-weekly intervals from October 2007 onwards, *i.e.* from the start of the rainy season in this summer rainfall area. The animals were scored according to the FAMACHA© system and individual anaemic animals were dewormed with 12 mg/kg levamisole. During the week of 21 January 2008, the experimental goats within each herd were ranked according to faecal egg count determined 2 weeks previously and the goats were randomly treated with 4 g COWP or not. Faecal egg counts were performed on the day of treatment and again 14 days later. The percentage reduction in egg counts as a result of the COWP treatment was determined according to the formula: $[1–(T2/T1)\times C1/C2)]\times 100$, where T denotes the mean egg count for the COWP-treated group and C the mean egg count for the untreated controls and the subscripts 1 and 2 denote pre- and post-treatment values, respectively. Symptomatic treatment was suspended 6 weeks prior to COWP administration and only goats for which the pre-treatment egg counts were 200 eggs per gram (epg) or more were used in the calculation. The mean pre- and post-treatment egg counts for the COWP-treated group ($n = 73$) were 2295 epg (range: 200–13300 epg) and 304 epg (range: 0–2833 epg). The corresponding values for the untreated controls ($n = 67$) were 2668 epg (range: 200–11533 epg) and 2624 epg (range: 0–14433 epg). A percentage reduction of 83 % was obtained. While post-treatment faecal cultures were not carried out, 72 % of the larvae cultured pre-treatment were *Haemonchus* spp. COWP show potential to be used as a tactical anthelmintic treatment to reduce the expected late-summer peak in faecal egg counts under these farming conditions.

Ticks of livestock in the North West Province of South Africa

A M Spickett and H Heyne

Parasites, Vectors and Vector-borne Diseases (PVVD), ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa. E-mail: spickettam@arc.agric.za

External parasites, more specifically ticks, as vectors of disease and agents of direct and indirect damage as well as production loss, often necessitating intensive control interventions, impact directly and indirectly on the macro- and micro economy of the livestock industry in southern Africa. A survey of livestock ticks occurring in the North West Province of South Africa was conducted to delineate distribution patterns, determine seasonality and establish disease relationships. Animal Health Technicians nominated by the Provincial Directorate of Veterinary Services conducted monthly collections of specimens at specified sites, from livestock hosts, in 3 phases in consecutive years from the northeastern, central and western regions of the Province. A 4th phase, simultaneous to and after collection had culminated, constituted the identification, database acquisition and distribution plotting of collected specimens by the ARC-OVI. A total of 1190 collections from 265 sites yielded 42 866 tick specimens from 7 Ixodid (*Boophilus* is here considered as a genus) and 3 Argasid genera, comprising 24 different tick species (20 Ixodids, 4 Argasids). The main Ixodid tick species collected were *Rhipicephalus evertsi evertsi* (31.5 %), *Hyalomma rufipes* (22.4 %), *Amblyomma hebraeum* (17.3 %), *Boophilus decoloratus* (12.5 %), *Rhipicephalus appendiculatus* (11.2 %), *Hyalomma truncatum* (2.3 %), *Boophilus microplus* (1.6 %), *Rhipicephalus evertsi mimeticus* (0.5 %), *Rhipicephalus simus* (0.21 %) and *Rhipicephalus sanguineus* (0.12 %). The North West Province of South Africa therefore harbours all of the major tick vectors of disease that occur in southern Africa, the major tick-borne diseases (*i.e.* heartwater, both African and Asiatic bovine babesiosis and anaplasmosis) being prevalent mainly in the northeastern region of the province which also displayed the highest tick species diversity. The vector of Asiatic bovine babesiosis and the vectors of bovine theileriosis are restricted to the northeastern region. The central region appears transitory to *A. hebraeum* and *B. decoloratus,* whilst the 2 *Hyalomma* vectors of Crimean Congo Haemorrhagic Fever virus are widespread over the whole province. The northwestern area (Bray) of the western region has been infiltrated by *R. evertsi mimeticus,* which is regarded as indigenous to Namibia. Serology conducted in conjunction with tick presence/absence data show most herds in the northeastern region to be endemically unstable to babesiosis and anaplasmosis and at risk to these tick-borne diseases should vector control measures become ineffective.

The effect of starvation on the fish ectoparasite *Dolops ranarum* (Crustacea: Branchiura)

Q Tam [1], A Avenant-Oldewage[1] & J Theron[2]

[1]*Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park, Johannesburg, 2006 South Africa. E-mail: aoldewage@uj.ac.za*
[2]*Aquaculture Research Centre, University of Limpopo, Private Bag X1106, Sovenga, 0727 South Africa*

The effect of starvation on the ultrastructure of the digestive cells of *Dolops ranarum,* a crustacean ectoparasite, was observed with the use of electron microscopy. Live specimens of *D. ranarum* were collected from Limpopo Province and the control specimens were immediately fixed in Todd's fixative, embedded in resin and sectioned. The experimental animals were allowed to starve and then were also fixed and sectioned. The digestive system of this parasite is composed of different cell types with various functions. The anterior midgut is composed of resorptive cells used to absorb nutrients while the enteral diverticula are composed of resorptive and fibril cells responsible for the production of enzymes. The posterior midgut is composed of mainly blister cells that are responsible for the digestion of waste material. The results of the experiment indicate that the anterior midgut and the enteral diverticula are the main storage areas for lipids. The number of lipid droplets is more numerous in fed specimens than in starved ones and also, the size of the cells as well as the diameter of the midgut viewed in cross section is smaller in starved specimens than in fed animals. The ability to store fats effectively allows this parasite to survive for up to 2 weeks without a host. This, amongst others, has implications on the period of time available to relocate a host after leaving it for egg deposition.

Software on-farm decision support systems required for sustainable anthelmintic efficacy

J A van Wyk

Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa. E-mail: jan.vanwyk@up.ac.za

Anthelmintic resistance has reached the level where on numerous farms in a number of countries even the most modern anthelmintics are severely affected, and there are none left that are 'fully' effective against the helminths of small ruminants any longer, while the helminths of cattle are rapidly following suit. Despite intensive efforts over decades to increase the sustainability of anthelmintic efficacy through alternative, integrated methods of parasite management (IPM), resistance is escalating unabated. In reaction, we helminthologists and advisors to farmers have endlessly been repeating much the same messages of IPM, with little adoption and uptake by farmers and even many of their advisors. Add to this a similarly severe and progressive reduction in trainers with experience, as well as some recent drastic changes in the approach to worm management and it is obvious that the chances of improving uptake by farmers is very unlikely to improve without radical changes to present methods of training and technology transfer. To find a solution to this problem has become more acute since announcement of a new anthelmintic activity group, the Amino-Acetonitrile Derivatives (AADs), to improve the chances that the mistakes of the past can be avoided. It is suggested that systems of targeted selective treatment, *i.e.* targeting treatment only at animals suffering from helminthosis instead of, as previously, treating all every time some take ill, are required for sustainability, but that even this can succeed only with use of systems of on-farm computerised decision support, which need to be developed.

South African Hexabothriidae: History, public aquarium importance, challenges, and the way forward

D B Vaughan[1] and K W Christison[2]

[1]*Two Oceans Aquarium, PO Box 50603, Victoria & Alfred Waterfront, Cape Town, 8000 South Africa. E-mail: dvaughan@aquarium.co.za*
[2]*Department of Environmental Affairs and Tourism, Marine and Coastal Management, Private Bag X2, Roggebaai, 8012 South Africa.*

The monogenean family Hexabothriidae Price, 1942 comprises 14 valid genera. These oligonchoinean monogeneans are sanguiniverous parasites of chondrichthyan fishes. Few published records exist for hexabothriid taxa in public aquaria. However, these records identify problematic taxa with emphasis on physical damage caused by feeding parasites in the gills, and the vulnerability of captive hosts to subsequent secondary bacterial infections. The successful management of hexabothriids in captive-held chondrichthyans is dependent on the availability of information of species associations between hosts and these parasites. Presently only 2 published descriptive records of South African hexabothriids exist, namely *Callorhynchocotyle callorhynchi* Manter, 1955, and *Branchotenthes robinoverstreeti* Bullard and Dippenaar, 2003. Both taxa are parasites of highly sought after chondrichthyan exhibit specimens in public aquaria. Since 2006, collections of hexabothriids from chondrichthyan fishes by-catch from the FRV Afrikana have indicated that South African hexabothriid diversity is high. However, the lower level taxonomy of the family is complicated by ambiguity in the nomenclature and inconsistencies within the literature. Herein we present an overview of the South African hexabothriid species diversity and biogeography within a global context.

Comparison of 4 light traps for the collection of *Culicoides* species (Diptera, Ceratopogonidae) under South African conditions

G J Venter[1], K Labuschagne[1], S N B Boikanyo[1] and E van den Berg[2]

[1]*Parasites, Vectors and Vector-borne Diseases (PVVD), ARC-Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort, 0110 South Africa. E-mail: venterg@arc.agric.za*
[2]*Biometry Unit, ARC-Institute for Agricultural Engineering, Private Bag X519, Siverton, 0127 South Africa*

Unpredicted outbreaks, spread and over wintering of bluetongue in Europe increased international interest regarding research on *Culicoides* midges as vectors of orbiviruses. The primary monitoring tools for collecting *Culicoides* midges are various models of light traps. To facilitate comparison of data 4 different downdraught light traps were compared under South African conditions. These were the Onderstepoort trap (220 V UV), the Rieb trap from France (12 V UV), the miniature CDC trap from the USA (6 V UV) and the Pirbright (220 V white light) trap from the United Kingdom. Traps were deployed in 3 replicates of a 4 × 4 randomised Latin square design, the advantage being that treatment means are independent of any effects due to site or occasion. Trapping was conducted on 12 nights in January 2008. In 48 collections made 643 374 *Culicoides* were collected. Eighteen different *Culicoides* species, of which only 6 were found in all 4 traps, were collected. All 4 traps indicated *Culicoides imicola* to be the most abundant species. Its abundance ranged from 91.8 % (Rieb) to 95.0 % (Onderstepoort). Significant differences were found in the average number of *Culicoides* collected per night by each of these traps. The Onderstepoort trap (average per night = 33 951) collected significant more midges than the CDC (13 733), Pirbright (3261) or Rieb trap (2670). Significant differences were also found in the parous rates, sex and *Culicoides* to other insect ratios as determined by the different traps. Although the Onderstepoort trap will increase monitoring sensitivity in areas where vector abundances are low it highlighted the notion that biases in trapping methods need to be evaluated and measured.

Comparison of insecticide resistance in larval and adult life-stages between 3 strains of the malaria vector, *Anopheles funestus*

O R Wood[1,3], B D Brooke[1,3], R H Hunt[1,2], L L Koekemoer[1,3], and M Coetzee[1,3]

[1]*Vector Control Reference Unit, National Institute for Communicable Diseases, NHLS, Private Bag X4, Sandringham, 2131 South Africa. E-mail: oliverw@nicd.ac.za*
[2]*School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Private Bag X3, WITS 2050, Johannesburg, South Africa*
[3]*Division of Virology and Communicable Disease Surveillance, School of Pathology of the National Health Laboratory Service and the University of he Witwatersrand, Johannesburg, South Africa.*

The development of insecticide resistance in malaria vectors poses a major problem for the control of both the pests and disease. The insecticide resistance levels of 3 strains of *Anopheles funestus* mosquito, FANG, FUMOZ, and FUMOZ-RH were examined. These strains exhibit full susceptibility, intermediate and high level resistance to pyrethroids, respectively. Permethrin coated bottles were used to assay the lethal dose resulting in 50 % mortality (LD_{50}) in adults, while cups containing a permethrin solution were used to assay 4th instar larvae. A comparative analysis between the adults showed that the strains were all significantly different from one another, with 14.6- and 76.2-fold increases for FUMOZ and FUMOZ-RH, respectively, in relation to FANG. This was repeated for the larvae where, in contrast to the adults, they did not differ significantly from each other. A further comparison was made between the life-stages for each of the strains. The only strain not exhibiting significant difference between its life-stages was FANG, whereas FUMOZ and FUMOZ_RH adults demonstrated 5.1 and 34.4-fold increases in resistance, respectively. These results suggest that the pyrethroid resistant phenotype is only exhibited in the adult life-stage. The difference in phenotypes between life-stages may need to be considered when choosing control interventions.

Poster Abstracts

Ecological parameters of an unidentified *Diplozoon* sp. on the bushveld smallscale yellowfish, *Labeobarbus polylepis* (Boulenger, 1907)

A Austin and A Avenant-Oldewage

Zoology Department, University of Johannesburg, PO Box 524, Auckland Park, 2006 South Africa. E-mail: aoldewage@uj.ac.za

The family Diplozoidae contains monogenean ectoparasites which occur on the gill lamellae of cyprinid fishes. Two hermaphroditic adult pairs fuse in permanent copulation to form this unique parasite. Very few species of the family Diplozoidae have been described from Africa. Monogeneans are notorious stenoxenic parasites frequently utilised for host species identification. Previous studies showed hybridisation of yellowfish species in Mpumalanga. The objective of this study was to identify a monogenetic parasite to aid host identification. Lowveld largescale yellowfish *Labeobarbus marequensis* (A. Smith, 1841), and bushveld smallscale yellowfish, *Labeobarbus polylepis* Boulenger, 1907 were collected during January 2007 and June 2008 from the Komati River, Mpumalanga, South Africa. The gills were examined for the presence of parasites with the aid of a dissection microscope. The position of attachment and region of the unidentified *Diplozoon* sp. on *L. polylepis* was noted. The specimens were collected from the gill filaments and fixed in warm acetoformaldehyde alcohol and preserved in 70 % ethanol. One *Diplozoon sp.* individual (prevalence 5.00 %, mean intensity = 1.00, abundance = 0.05) was collected from 20 fish in January 2007 and 3 individuals (prevalence 7.41 %, mean intensity = 1.50, abundance = 0.11) were collected on 27 fish in June 2008 in the Komati River. The *Diplozoon sp.* occurred on only 1 host species. This study contributed to the distribution range of monogeneans in southern Africa. Furthermore, it was established that the very low level of the parasite infection renders this parasite unsuitable as a host species identification instrument.

Preliminary studies on oviposition site preferences of *Culicoides imicola* (Diptera: Ceratopogonidae)

S N B Boikanyo and G J Venter

Parasites, Vectors and Vector-borne Diseases (PVVD), ARC-Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort, 0110 South Africa. E-mail: boikanyos@arc.agric.za

Light trap collections have shown *Culicoides imicola,* considered a vector of both African horse sickness and bluetongue virus, to be the most widespread and abundant livestock associated *Culicoides* species in South Africa. Despite this, relatively little is known about the biology of this *Culicoides* species. A laboratory study was undertaken to clarify the oviposition preference of *C. imicola.* Field collected midges were fed on defribrinated ovine blood. Blood engorged females were offered a choice of differently treated oviposition surfaces. The artificial oviposition device consisted of a plastic Petri dish (ø = 35 mm) with a double layer of filter paper on top of tamped-down moist cotton wool on the bottom of the holding container. This provided an even surface on which eggs could be laid. Salt concentrations varying from 0.003 to 3.0 g/10 mℓ and infusions of sheep, horse and bovine dung were compared. In an additional treatment, engorged females were given a choice between oviposition surfaces heated to 22 °C and 25 °C. All treatments were done in duplicate. Flies were kept at 23.5 °C and the 1st eggs were usually deposited after 3 days. It was shown that *C. imicola* prefer oviposition sites with a salt concentration below 0.06 g/10 mℓ. Extracts of horse dung were preferred and increase the notion that horses are the preferred host of *C. imicola.* It was also found that there was a preference to the 25 °C surface and this supports the idea that *C. imicola* will rather oviposit in areas heated by direct sunlit than in shaded areas

A novel *Babesia* sp. found in cheetahs (*Acinonyx jubatus*) in South Africa

A-M Bosman, M C Oosthuizen, E H Venter and B L Penzhorn

Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa. E-mail: bossie.bosman@up.ac.za

The cheetah is listed in Appendix I (most threatened species) in the World Conservation Union (IUCN) Red List of Threatened Species (2008). This is mainly because of loss of habitat in the wild and conflicts with farmers in remaining habitats. Between 12 000 and 15 000 cheetahs remain in the wild, mostly in small-pocketed populations in 24 to 26 countries in Africa. Cheetahs are also suspected to be particularly vulnerable to infectious diseases. Both small and large intraerythrocytic piroplasms have been reported from a variety of domestic and wild felid species from several continents. However, not much is known about the prevalence of these piroplasms in cheetahs. In a recent survey, it was found that *Babesia felis* and *B. leo* do occur in cheetahs, but not as mixed infections. A large number (52.9 %) of cheetahs were infected with an as yet undescribed *Babesia* species. The aim of the current study was to characterise this undescribed *Babesia* sp. by phylogenetic analysis based on the 18S rRNA gene and the 2nd internal transcribed spacer region (ITS-2) sequences. A BLAST search performed with the obtained 18S rRNA gene sequence revealed no identical sequences in the public databases and it was designated *Babesia* sp. (cheetah). The most closely related sequence (~96 % identity) was from *Babesia conradae* (AF231350 and AF158702), previously identified from dogs in California, USA. The ITS-2 sequence data as well as phylogenetic analysis confirmed these results.

The ultrastructure of the mange mite *Psoroptes pienaari* (Fain, 1970) occurring on the African buffalo *Syncerus caffer*

F C Clarke[1], M L Turner[2] and E A Ueckermann[3]
[1]*Department of Biology, Medunsa Campus, University of Limpopo, 0204 South Africa. E-mail: clarke@ul.ac.za*
[2]*Electron Microscope Unit, Medunsa Campus, University of Limpopo, South Africa*
[3]*ARC-Plant Protection Research Institute, Pretoria, South Africa*

Psoroptes pienaari abrade the surface of the skin and feed on the exudate, epidermal debris and lipids thus aquired, causing inflammation and hypersensitivity. This leads to psoroptic mange in the African buffalo. The classification of the different host specific species of *Psoroptes* is based mainly on the host species, predilection site and morphological characters of adult male mites as seen through the light microscope. Zahler *et al.* (2000) compared 5 species, *P. caniculi, P. ovis, P. equi, P. cervinus* and the cattle mange mite *P. natalensis* and concluded that the features used to discriminate between the species are not unequivocal and regarded these species as synonyms of *P. equi.* An improved knowledge of the ultrastructure of the different species might prove invaluable in determining species status of the mange mites found on the different hosts. Live mites were collected from an African buffalo and fixed in 70 % ethanol. Samples were routinely processed for scanning electron microscopy and viewed in a Leica Stereoscan 420 scanning electron microscope at 5–7 kV. The taxonomically important structures of both male and female tritonymphs and adults of *P. pienaari* were investigated. These included the anus, reproductive apparatus, opisthosomal lobes, adanal suckers and relevant setae in the males as well as the dorsoposterior tubercles of the female tritonymph and the reproductive apparatus and anus of the adult female. Special attention was also given to the ambulacral structures of the jointed pretarsi and the mouthparts.

The relevance of *Glossina austeni and Glossina brevipalpis* (Diptera, Glossinidae) colonies for tsetse fly research and management in South Africa

C J de Beer, G J Venter and A A Latif
Parasites, Vectors and Vector-borne Diseases (PVVD), ARC-Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort, 0110 South Africa. E-mail: debeerc@arc.agric.za

Tsetse eradication is assuming increasing importance as it has become evident that the tsetse and trypanosomosis is a contributing factor to perpetuating rural poverty within Africa. The adoption of an area-wide approach for integrated pest management where conventional methods are used in combination with the Sterile Insect Technique (SIT) to eliminate tsetse can have a major impact on socio-economic development by increasing land available for agricultural exploitation and increased livestock development. A prerequisite for the use of SIT is the colonisation and mass rearing of the target insect species. *Glossina brevipalpis* and *Glossina austeni* are vectors of nagana in animals in northeastern KwaZulu-Natal in South Africa. Both species occur mainly in the game reserves and rural communities close to the reserves. Colonies of *G. brevipalpis* and *G. austeni* have been established in 2002 at the ARC-OVI from seed material obtained from TTRI, Tanga (Tanzanian) and IAEA tsetse colonies at Seibersdorf (Austria), respectively. Flies are maintained on bovine blood obtained from local abattoirs. Both males and females are fed on an *in-vitro* system with the use of silicone membrane. Tsetse flies are ovoviviparous and each female only produce a single larva every 10 days after mating. Productive females are kept for a maximum of 12 weeks in the colony. The pupal stage can be up to 30 days at 25 °C and a relative humidity of 75 %. Despite this relatively low production rate colonies are currently being maintained at 18 591 and 25 584 productive females for *G. brevipalpis* and *G. austeni*, respectively.

The evaluation of the susceptibility of *Trypanosoma congolense* isolates collected from cattle and buffalo in KwaZulu-Natal to isometamidium chloride and diminazene aceturate

J F du Plessis[1], J Masumu[1], R Nkuna[1], B L Penzhorn[1], P T Matjila[1] and P Van Den Bossche[1,2]
[1]*Department of Veterinary Tropical Diseases, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa. E-mail: duplessis.f@gmail.com*
[2]*Institute of Tropical Medicine, Animal Health Department, Nationalestraat 155, B-2000 Antwerp, Belgium*

To assess the occurrence of resistance to trypanocides in KwaZulu-Natal, a total of 11 *Trypanosoma congolense* Savannah-type isolates were collected and characterised in mice using the single-dose protocol. These isolates were collected from cattle ($n = 6$) and African buffaloes ($n = 5$). For each isolate, 3 groups of 6 mice were inoculated intraperitoneally with 10^5 trypanosomes in 0.2 mℓ of a PSG solution. Twenty-four hours later, mice belonging to 2 groups were treated intraperitoneally with a solution of isometamidium chloride (ISM, 1 mg/kg) and diminazene aceturate (DA, 20mg/kg), respectively. The 3rd group was kept as control. The presence of parasites in the blood was checked twice a week by microscopical examination of a drop of blood collected from the tail. Mice were observed for relapse up to 60 days post-infection. Relapses were observed in single mice in 4 groups, *i.e.* infected with 4 different isolates, respectively. One isolate originated from cattle and 3 from buffalo. One relapse was observed in the ISM-treated group (cattle isolate) and 3 in the DA-treated group (buffalo isolates). According to the protocol used, an isolate is considered resistant when at least 2 of the 6 mice relapse. Since only 1 relapse was observed from each of the 4 isolates, the 11 isolates tested were all considered to be sensitive to both drugs. The results of this study suggest either drug resistance is not present in the study area or the prevalence of isolates that are resistant to either DA or ISM is very low.

A new *Gnathia* sp. (Crustacea: Isopoda: Gnathiidae) from Lizard Island, Great Barrier Reef, Australia

C Farquharson[1], N J Smit[1], A S Grutter[2] and A J Davies[1,3]
[1]*Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park, 2006 South Africa. E-mail: aquarium@uj.ac.za*
[2]*School of Integrative Biology, University of Queensland, Brisbane, Queensland, 4072, Australia*
[3]*School of Life Sciences, Faculty of Science, Kingston University, Kingston upon Thames, KT1 2EE, United Kingdom*

Gnathiids are marine isopods with a worldwide distribution, and the majority of species are described from temperate and tropical waters. These organisms are unique among isopods in being protelian parasites with only five pairs of walking legs. The parasitic juvenile stages feed on a wide variety of fish hosts, including elasmobranchs and teleosts. As part of a gnathiid biodiversity study on the Great Barrier Reef (GBR), samples were collected with small light traps at Casuarina Beach and Coconut Beach, Lizard Island,

Australia, in November 2005. Trap samples were sorted and among the specimens were males, females and juveniles of a species that did not conform in appearance to any known gnathiid. Since the taxonomy of these isopods is based on the morphology of adult males, final stage juveniles were allowed to moult into further adults. Males of the new *Gnathia* sp. are characterised by a shallow dorsal sulcus that stretches almost the entire length of the cephalosome; the cephalosome also has a non-produced frontal border with 2 conical superior fronto-lateral processes and a conical inferior medio-frontal process. The mandibles have 6 processes on each dentate blade and a distinct internal lobe with 8 processes. The male of this species can be distinguished from most other GBR gnathiids by the internal lobe on the mandible, which is absent in the majority. Although *Gnathia falcipenis* and *Gnathia cornuta* from the region have an internal lobe, they also have different shaped frontal border processes from the new species.

The micromorphology of the *Menacanthus* body lice collected from a partridge.

E D Green[1] and M L Turner[2]
[1]*Department of Anatomy, University of Limpopo (Medunsa Campus), Box 232, Medunsa, 0204 South Africa. E-mail: edward@ul.ac.za*
[2]*Electron Microscopy Unit, University of Limpopo (Medunsa Campus), Box 232, Medunsa, 0204 South Africa.*

Lice collected from a partridge at a veterinary clinic in Dubai were identified as a species of *Menacanthus.*With more than 60 reported species of these bird lice, it was decided to do a scanning electron microscope (SEM) study in order to determine micromorphological characteristics of this species. The lice were ultrasonically cleaned and routinely prepared for SEM and viewed in a Leica 420 Stereoscan at 5.8 kV. The shovel-shaped head bore the following structures typical of the family Menoponidae; 4-segmented maxillary palpi with 11 distal sensilla and labial palpi surrounding the oral cavity; and 4-segmented pedunculate antennae with distal sensilla, which folded into an antennal groove on the lateral sides of the head. A pair of prominent spinelike processes posterior to the oral cavity may be used to rip the skin to allow the blood flow for ingestion. This may account for the anaemia observed in the partridge. The thorax segments were not fused and their shape and distribution of setae was closer to *Menacanthus cornutus* than the common chicken body louse *M. stramineus.* The 3 pairs of legs each ended in an elongated tarsus with a small padlike process, and 2 long hooked tarsal claws. These claws were well-ridged which increased the potential to grasp the feathers of the host. The abdominal segments had rows of short setae medially and long setae and round spiracles laterally. The female gonopods XI had a posterior-medial flap, lined with a fringe of setae, which may stabilise the egg during ovipositing. These observations may be of morpho-taxonomic importance in distinguishing closely related *Menacanthus* species.

Gertrud Theiler Tick Museum, Onderstepoort Veterinary Institute, South Africa

H Heyne and A Latif
Parasites, Vectors and Vector-borne Diseases (PVVD), ARC-Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort, 0110 South Africa. E-mail: heyneh@arc.agric.za

The OVI houses the Gertrud Theiler Tick Museum, previously known as the African Tick Museum. The National Tick Collection was started by G A. Bedford in 1912 with *Aponomma exornatum* nymphs collected from a water leguaan (*Varanus niloticus*) in the Onderstepoort locality. From 1942 to 1967 the collection was largely extended by Gertrud Theiler (1939-1967). During the past century various other tick workers have also contributed toward the collection: J B Walker, M B Baker, J D Bezuidenhout, A M Spickett, I H Horak, A Latif, H Heyne, and I McKay to name a few. Various other acarologists such as Kolonin from Russia; Tenderoi from Portuguese Africa; Hoogstraal from the USA Navy Laboratories in Cairo; Estrada-Peña from Spain; Frans Jongejan from Utrecht and others have also exchanged tick specimens and numerous species have been added to the National Tick Collection. This collection is regarded as a national asset and is now one of the largest collections of ticks in the world. The Gertrud Theiler Tick Museum comprises more than 3000 collections, mainly from South Africa, but even as far as Nepal. Nearly all the widely known tick genera are represented in the Museum comprising 338 species with 43 type specimens, consisting of holotypes, allotypes and paratypes. Almost the entire genera of *Rhipicephalus* and *Hyalomma* of the world are contained in this collection with 74 species out of the recorded 87 and 24 out of 30, respectively. The Museum also contains the world rare species: *Nosomma monstrosum, Cosmiomma hippopotamensis,* all species in the genera *Margaropus* and *Rhipicentor,* and the evolutionary 'missing link' *Nuttalliella namaqua.* The intention is to use the Tick Museum 1stly as a functional taxonomic reference centre for African ticks, vital to continuing biosystematic studies of these economically important parasites and secondly, as a training centre for tick ecology and epidemiology for tick workers in Africa and the world.

Spatial distribution of blackfly (Diptera: Simuliidae) challenge for livestock farmers along the Vaal River, South Africa

V L Hobololo[1,2]
[1]*Parasites, Vectors and Vector-borne Diseases (PVVD), ARC-Onderstepoort Veterinary Institute, Private Bag X 05, Onderstepoort, 0110 South Africa. E-mail: hobololov@arc.agric.za*
[2]*Department of Veterinary Tropical Diseases, University of Pretoria, Faculty of Veterinary Sciences, Private Bag X04, Onderstepoort, 0110 South Africa*

In South Africa, blackflies have been implicated in the spread of 2 pathogens, namely the *Chlamydia* species and Rift Valley Fever virus which cause blindness in sheep and abortion in small ruminants and cattle, respectively. Field trials for testing potential control measures for this insect pest of veterinary significance can only provide meaningful data for practical application within the context of integrated pest management when conducted in areas of proven blackfly challenge. A total of 100 farms along the Vaal river were surveyed for this study, and their locality was recorded by map co-ordinates using GPS. The data were analysed with the use of ArcGIS version 9, a geographical information systems program. The infestation indices differed significantly among the 5 provinces across which the Vaal river runs. However, there was positive correlation between high incidence of blackfly infestation which in other areas (Christiana and Bloemhof) manifested in the form of outbreaks and close proximity to river bank in areas where rapids were abundant. The Mpumalanga province proved to be the least affected as it had less incidences of blackfly infestation, while Northern Cape and North West were severely affected. The areas in the latter provinces where the livestock industry was highly threatened were identified as appropriate locations to test different control measures aimed at intercepting the adult stage of the blackfly.

Preliminary survey of the presence of intestinal *Eimeria* spp. in domestic bovines, ovines and caprines from selected sites in South Africa

R Jooste[1], P Page[1] and P Qwalela[1]
[1]*Bayer Animal Health, P. O. Box 143, Isando, 1600 South Africa. E-mail: jooste@bayerhealthcare.com*

Eimeria spp. are important pathogens of domestic livestock. Disease is characterised by enteritis and is pertinent on farms where animal densities are high, or hygiene is poor with accompanying high stress levels. Economic losses are suffered through death, poor production, costs of treatment and prophylactic management. These are particularly relevant in feedlots and intensive juvenile rearing systems when effective diagnosis and treatment may be delayed. The most important pathogenic species are regarded as *Eimeria zuernii, E. bovis* and *E. aubernensis* in cattle, *E. bakuensis, E. ahsata* and *E. ovinoidalis* in sheep; and *E. arloingi, E. christenseni, E. caprovina* and *E. ninakohlyakimovae* in goats. Mixed infestations, with less pathogenic species, exacerbate the situation. Faecal samples were collected from animals, 1–9 months of age, in 40 districts in South Africa. Samples were refrigerated, transported to independent diagnostic facilities, and enumerated using a Modified McMaster Technique. A total of 56 bovine, 78 ovine, and 22 caprine samples were examined for the presence of oocysts. In bovines *E. zuernii, E. bovis, E. ellipsoidalis,*

E. cylindrica, E. canadensis, E. alabamensis, E. pellita, and *E. wyomingensis* were identified. In ovines *E. bakuensis, E. ahsata, E. ovinoidalis, E. crandallis, E. parva, E. faurei, E. intricata, E. weybridgensis* and *E. granulosa* were identified. In caprines *E. arloingi, E. christenseni, E. caprina, E. jolchijevi, E. aljevi* and *E. hirci* were identified. Sixteen (28.6 %) bovine, 51 (65.4 %) ovine and 14 (63.6 %) caprine samples were positive for *Eimeria* spp. Fifteen (26.8 %) bovine, 49 (62.8 %) ovine and 13 (65.0 %) of the caprine samples, had a history of diarrhoea and were positive for *Eimeria* spp. The survey confirms the presence of pathogenic intestinal *Eimeria* spp. in domestic bovines, ovines and caprines on commercial farms in South Africa.

A review of zoonotic importance of leishmaniosis: Significance of diagnosis in South Africa

T C Katsande, N Letsoalo, B Peba, O Matthee and E Zweygarth.

Parasites, Vectors and Vector-borne Diseases (PVVD), ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa. E-mail: mabogoanen@arc.agric.za

Leishmaniosis is a zoonotic disease affecting many vertebrate hosts including man and dogs. In areas where the disease is endemic, it is of major public health concern and various forms of clinical manifestations of human leishmaniosis have been described. The increasing international travel by people and their pet dogs has increased demand for reliable and accurate diagnostic tests by the Department of Veterinary Services. South Africa has been reported to be free from the disease. The epidemiology of the disease in the world and the risk of introduction into South Africa are discussed. The Commercial test kit (CIVTEST™ CANIS LEISHMANIA (HPRA)), which is a Membrane Immunoassay based on the Indirect ELISA principle was routinely used for testing dogs for importation purposes in South Africa up until 2006. A comparison of the 2 tests showed that the CIVTEST has a sensitivity of 100 % and a specificity of only 81 % as compared to the IFAT. The observed agreement between the 2 tests was 88 % with a KAPPA value of 0.77, indicating a good but not perfect agreement. The CIVTEST therefore has a limitation of low specificity and the IFAT was recommended by the National Department of Veterinary Services, South Africa, for routine testing of dogs for importation purposes to replace the CIVTEST. A review of the 2 tests is presented including the merits and demerits of the tests. The significance of current diagnostic techniques and interpretation of the test results in preventing the introduction of leishmaniosis into South Africa are discussed.

The insect reference collection at the ARC-OVI

K Labuschagne

Parasites, Vectors and Vector-borne Diseases (PVVD), ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa. E-mail: labuschagnek@arc.agric.za

The insect reference collection of the ARC-OVI is a national asset and is used as an educational tool for students and visiting researchers. The collection consists mainly of Diptera species although some other genera are represented as well. Other genera include Coleoptera, Lepidoptera, Neuroptera and Siphonaptera. The Diptera collection includes: *Musca* spp., *Stomoxys* spp., *Glossina* spp., mosquito (*Anopheles* spp., *Aedes* spp., *Culex* spp.), and *Culicoides* spp. The collection is divided into dry pinned, slide mounted and alcohol specimens. The pinned collection is housed in 1 large cabinet consisting of 42 drawers and 2 smaller cabinets with 10 drawers each. Specimens, with their collection details, are stored in small separate boxes according to their genus. The slide collection consists of some 12 000 microscope slides from 190 localities and 720 species. The species include 207 *Culicoides* and 513 other Nematocera species, such as *Forcipomyia* spp., *Dasyhelea* spp., *Bezzia* spp. and *Atrichopogon* species. The insect reference collection stored in alcohol mainly consists of some 10 800 *Culicoides* collections. The date, collection site/area, total number of *Culicoides* in the collection as well as species breakdown (female: nulliparous, parous, gravid, blood-fed and males) are recorded on both an access database and a file system. The insect reference collection is used for identification, comparison, description of new and redescription of old species. Digital photographs are taken of slide mounted *Culicoides* species. These photographs are used for description of species and to compile a new updated wing photo atlas of South African *Culicoides* species.

Metazoan parasites of *Micralestes acutidens* from the Limpopo River drainage system, South Africa

P K Ledwaba[1], J Theron[2] and W J Luus-Powell[3]

[1]*Limpopo Department of Agriculture, Tompi Seleka College, Private Bag X9619, Marble Hall, 0450 South Africa. E-mail: patric_7610@yahoo.com*
[2]*School of Agricultural and Environmental Sciences, Aquaculture Research Unit University of Limpopo (Turfloop Campus), Private Bag X1106, Sovenga, 0727 South Africa*
[3]*School of Molecular and Life Sciences, Department of Biodiversity, University of Limpopo (Turfloop Campus), Private Bag X1106, Sovenga, 0727 South Africa*

The silver robber (*Micralestes acutidens*) is a member of the Characidae with economic value as an aquaculture species. The silver robber is considered a potential aquarium species due to its shiny colour and small size. The health and parasitic composition were investigated at the Luphephe-Nwanedi Dam (Limpopo River system, Limpopo Province). Four surveys were conducted from May 2007 to April 2008 and a minimum of 20 fish of different sizes were collected during each survey using seine, cast nets and an electric shocker. The parasites were fixed with hot alcohol-formalin-acetic acid (AFA) and stored in 70 % ethanol and some monogeneans were mounted in glycerine jelly. The following parasites were recorded: *Annulotrema* sp. from the gills (prevalence of 13.5 % and abundance 0.43); *Diplostomum* larvae from the eyes (prevalence of 16 % and abundance 0.8); gryporhynchid cestode larvae embedded in the intestine layer (prevalence of 7.4 % and abundance 0.16) and small *Contracaecum* larvae from the body cavity (prevalence of 6.2 % and abundance 0.09). A single *Afrogyrodactylus* sp. specimen was recovered from the gills which represents the 1st record of this genus from South Africa.

An endoparasitic monogenean from *Oreochromis mossambicus*

G N Madanire[1], P A S Olivier[1], M M Matla[1], J Theron[2] and W J Luus-Powell[1]

[1]*Department of Biodiversity, School of Molecular & Life Sciences University of Limpopo (Turfloop Campus), Private Bag X1106, Sovenga, 0727 South Africa. E-mail: gracemad@webmail.co.za*
[2]*Aquaculture Research Unit, University of Limpopo (Turfloop Campus), Private Bag X1106, Sovenga, 0727 South Africa*

Monogeneans are usually ectoparasites and found on the gills and skin of their host. Amongst the Dactylogyridea, some show unusual localisation like the urinary bladder, ureters, nasal cavity and oesophagus. During parasitological investigations of the Mozambique tilapia in the Luphephe-Nwanedi Dam (22°39′S 30°25′E), Limpopo River system (South Africa), a monopisthocotylean monogenean of the genus *Enterogyrus* Parpena, 1963 was found in the stomach of the host. This genus currently includes 7 species which are predominantly parasites of the stomach of tilapine cichlids. *Enterogyrus cichlidarum* was, however, also recorded from the liver, heart and blood vessels of juvenile Mozambique tilapia from America (introduced with their host). Fish were collected from May 2007 to July 2008 using gill nets of various mesh sizes and killed by severing the spinal cord. The gastrointestinal tract was dissected and parasites encountered were carefully detached from the stomach mucosa. The monogeneans were fixed with hot alcohol-formalin-acetic acid (AFA) and stored in 70 % ethanol or mounted in glycerine jelly. Six of the 41 hosts examined were infected and a total of 45 parasites were collected (prevalence 14.6 %; intensity range 1–17; mean intensity 7.5; abundance 1.09). All previous records of *Enterogyrus* are from northern and western Africa (Egypt, Cameroon, Senegal and Ivory Coast) and recently also from introduced cichlids from Mexico. This study represents the 1st record of an *Enterogyrus* species from South Africa.

Monogeneans of some fish species in the Luphephe-Nwanedi Dam, South Africa

E M Mbokane[1], G N Madanire[2], W J Luus-Powell[2], M M Matla[2] and J Theron[1]

[1]*Aquaculture Research Unit, University of Limpopo (Turfloop Campus), Private Bag X1106, Sovenga, 0727 South Africa. E-mail: 200307054@ul.ac.za*
[2]*Department of Biodiversity, University of Limpopo (Turfloop Campus), Private Bag X1106, Sovenga, 0727 South Africa*

Freshwater fish in all inland waters in Africa are susceptible to infection by monogeneans that are host specific. Several monogenes were collected from the skin and gills of various fishes from the Luphephe-Nwanedi Dam (22°39'S, 30°25'E), Limpopo River system. Hosts were collected using gill nets. The monogenean parasites were fixed and mounted using alcohol-formalin-acetic acid (AFA) and glycerine jelly according to the standard methods of preparation for microscopic analysis. A total of 173 fish specimens comprising of *Oreochromis mossambicus* (n = 41), *Clarias gariepinus* (n = 38), *Labeobarbus marequensis* (n = 24), *Barbus trimaculatus* (n = 18), *B. radiatus* (n = 40) and *Schilbe intermedius* (n = 12) were examined from May 2007 to July 2008. Most monogeneans were found on the gills except *Gyrodactylus rysavyi* and *Macrogyrodactylus congolensis*, which are from the skin. Each of these monogenean flatworms has its unique and distinct opisthaptor with hardened anchors and marginal hooks that pierce the epithelium and hold on to the host. *Cichlidogyrus halli* (prevalence 70 %, mean intensity 4.3) collected from *O. mossambicus* had the highest prevalence. *Macrogyrodactylus clarii* (prevalence 31.6 %, mean intensity 1.4), *M. congolensis* (prevalence 15.8 %, mean intensity 1.8), *G. rysavyi* (prevalence 34.2 %, mean intensity 1.4) and *Quadriacanthus* sp. (prevalence 13.2 %, mean intensity 3.2) were recorded from *C. gariepinus*. *Neodiplozoon polycotyleus* infected both *B. trimaculatus* (prevalence 22 %, mean intensity 1.75) and *B. radiatus* (prevalence 37.5 %, mean intensity 2.1) whilst *Dactylogyrus spinicirrus* (prevalence 28 %, mean intensity 2.6) was found on the gills of the latter 2 hosts and *Labeobarbus marequensis*. *Schilbetrema quadricornis* (prevalence 50 %, mean intensity 34.5) was the numerically dominant species and recorded from the gills of *Schilbe intermedius*.

The African clawed frog (*Xenopus laevis*): a haven for parasites

E M Mbokane[1], J Theron[1] and W J Luus-Powell[2]

[1]*Aquaculture Research Unit, University of Limpopo (Turfloop Campus), Private Bag X1106, Sovenga, 0727 South Africa. E-mail: 200307054@ul.ac.za*
[2]*Department of Biodiversity, Private Bag X 1106, University of Limpopo (Turfloop Campus), Sovenga, 0727 South Africa*

The African clawed frog is aquatic and native to sub-Saharan Africa. They harbour a diverse parasite fauna with most species unique to this host. Because of its high abundance and usefulness in research studies and as a model organism for cell and developmental biology, the parasites of this frog have been studied extensively. However, little information is available from feral populations from the Limpopo Province. A total of 78 *Xenopus laevis* (mean snout–vent length, 44 ± 21 mm SD) were collected from 3 different locations in the Limpopo Province and were killed with an overdose of MS-222. The following parasites were found: *Trichodina xenopodos* (Protozoa) and *Protopolystoma xenopodis* (Monogenea) from the urinary bladder; 2 species of Digenea from the intestine, *Progonimodiscus doyeri* and *Dollfuschella rodhaini*; *Cephalochlamys namaquensis* (Cestoda) from the intestine and 2 species of Nematoda, *Camallanus xenopodis* from the intestine and *Batrachocamallanus slomei* from the stomach. The infestation statistics of all parasites are presented and discussed.

Development of a duplex real-time RT-PCR for detection and identification of BTV and AHSV

L Modibedi[1,2] and J J O Koekemoer[1,2]

Molecular Epidemiology and Diagnostics Programme, ARC-Onderstepoort Veterinary Institute, Onderstepoort, 0110 South Africa. E-mail: modibedil@arc.agric.za
[2]*Department of Veterinary Tropical diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.*

African horse sickness (AHS) and bluetongue (BT) are notifiable to the OIE because of their severe economic consequences and rapid spread. These diseases are caused by 2 different orbiviruses, African horse sickness virus and bluetongue virus belonging to the family *Reoviridae*. Both viruses are transmitted between their mammal hosts by biting midges of the genus *Culicoides* (Diptera, Ceratopogonidae) and in South Africa in particular by *Culicoides imicola*. Rapid and reliable diagnosis is of importance in the implementation of effective control measures. Real-time PCR represents a powerful tool for molecular diagnostics because this technology is very rapid and avoids contamination. In this study, we describe a duplex real-time RT-PCR using the fluorogenic dye SYBR® Green I for the specific detection and identification of both AHSV and BTV in 1 reaction. Differentiation between the viruses is based on melting temperature(T_m) analysis of the amplification products. Two primer pairs were designed to bind to areas of homology within segment 7 (VP7) of AHSV and segment 5 (NS1) of BTV. When in duplex, 2 melting peaks were simultaneously generated at 76.30 °C and 80.04 °C representative of BTV and AHSV, respectively. Serogroup-specific products were amplified from dsRNA of field isolates of AHSV and BTV. dsRNA from EHDV and EEV failed to demonstrate either the 232 bp specific AHSV PCR product or the 79 bp specific BTV product. These results indicate that the duplex real-time RT-PCR could be very useful as a single method that could be used for the detection of both AHSV and BTV from clinical samples used for diagnosis of either of the diseases.

Cichlidogyrus spp. Paperna, 1960 on the gills of *Tilapia rendalli* from the Okavango Delta, Botswana

E M Modise[1], P H King[1] and C Baker[2]

[1]*Department of Biology, University of Limpopo, Medunsa Campus, South Africa. E-mail: lizzymodise@yahoo.com*
[2]*Electron Microscope Unit, University of Limpopo, Medunsa Campus, South Africa*

Cichlidogyrus species are gill parasites with an extremely high degree of host specificity within the family Cichlidae. The genus *Cichlidogyrus* is represented by 65 species, which have all been described from Africa. The objectives of this investigation were to identify and study the morphology of monogenean parasites on *Tilapia rendalli* (redbreast tilapia) from the Okavango Delta, Botswana. Fish were collected from different localities in the Delta using gill nets. Collected fish were anaesthetised with clove oil, identified, measured and the gills removed. Gills were examined for monogeneans using a dissection microscope. These parasites were collected and mounted in ammonium picrate whereafter the opisthaptoral armatures and reproductive organs were studied through light microscopy. Four monogenean species of the genus *Cichlidogyrus* Paperna, 1960 were found to infest *T. rendalli* namely: *Cichlidogyrus halli* (Price & Kirk, 1967), *Cichlidogyrus sclerosus* (Paperna & Thurston, 1969), *Cichlidogyrus dossoui* Douëllou, 1993 and *Cichlidogyrus quaestio* (Douëllou, 1993). These 4 species showed distinct variations of the attachment structures in the opisthaptor, especially the shape of the anchors and bars. The most characteristic differences are the shape of the copulatory organ and the vagina. This study produced 4 different monogenean species from the redbreast tilapia in the Okavango Delta in Botswana.

Digenetic trematodes of marine fish in the Tsitsikamma National Park

P H King and E B E Moema

Department of Biology, PO Box 139, University of Limpopo, Medunsa Campus, 0204 South Africa. E-mail: ping@ul.ac.za

In a freshwater environment digenean parasites are known to use fish as 2nd intermediate hosts. Fish sampled in the Tsitsikamma National Park were found to be infected with several species of trematodes, all of which were adults. The objective of this study was to study these parasites through light microscopy. Fish were collected using hand line and scoop nets. Back in the field laboratory they were euthanised using clove oil, dissected and examined for parasites that were fixed in 70 % etOH for light microscopy. They were later stained with Van Cleave's haematoxylin. This poster reports on the intestinal infections of 4 fish species. *Clinus*

superciliosus (highfin clinid) was found to be infected with 4 different trematodes. Two of these were identified as *Coitocaecum capensis* and *Helicometra fasciata,* respectively. The remaining species are still unknown. *Diplodus capensis* (blacktail seabream) was also found to host 4 trematode species. One was identified as an Aspidogastrean species, possibly *Cotylogaster basiri,* the 2nd resembles *Steringotrema pagelli,* while the remaining are unknown. *Sarpa salpa* (strepie) was infected with 2 trematodes, of which one was identified as *Elstia stossichianum.* The 2nd species is unknown. *Sparodon durbanensis* (white musselcracker) was also found to host 2 trematodes, of which 1 was identified as *Pachycreadium obovatum.* The 2nd species is unknown. From this study it is clear that a diverse group of trematodes parasitise the intestines of small marine fish species. The taxonomy of these parasites and the intermediate hosts in their life cycles are unknown and should receive attention.

An update of the bovine trypanosomosis situation at the edge of Hluhluwe-Imfolozi Park, KwaZulu-Natal Province, South Africa

R Nkuna[1], J Esterhuizen[1], P T Matjila[1], B L Penzhorn[1], S Geerts[2], T Marcotty[2] and P Van Den Bossche[1,2]

[1]*Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa. E-mail: rhulani.nkuna@up.ac.za*
[2]*Institute of Tropical Medicine, Animal Health Department, Nationalestraat 155, B-2000 Antwerp, Belgium*

In South Africa, the distribution of tsetse has undergone considerable changes over the last century. Since the nagana outbreak of the 1990s little information is available on the prevalence of the disease in cattle. The aim of this study was to obtain updated data on and assess the contribution of trypanosomosis to the disease burden of cattle kept at the edge of the Hluhluwe-Imfolozi Park, KwaZulu-Natal Province. A survey was conducted at Mvutshini dip tank adjacent to the northern edge of the tsetse-infested area. Use was made of a purposeful sampling strategy by restricting sampling to animals that the livestock owner considered to be in poor condition. A total of 76 adult (12 months of age) cattle (Angoni breed) were sampled. From each animal, jugular blood was collected in vacutainer tubes coated with EDTA. Molecular (PCR-RFLP) and parasitological techniques were used to analyse the samples. Twenty-six (34.2 %) of the samples were parasitologically positive and 46 (60.5 %) were positive on PCR-RFLP. All parasitologically positive animals were also positive on PCR-RFLP. Almost all infections were due to *Trypanosoma congolense* Savannah subgroup. The average packed-cell volume (PCV) of all animals sampled was 19.8 ± 4.2 %. The average PCV of parasitologically positive animals (18.6 ± 3.8 %) differed little ($P > 0.05$) from the average PCV of parasitologically negative animals (20.5 ± 4.4 %). Similarly, the average PCV of animals positive on PCR-RFLP (19.6 ± 4.3 %) differed little ($P > 0.05$) from the average PCV of animals negative on PCR-RFLP (20.2 ± 4.3 %). A total of 63 animals had a PCV ≤ 24 %. This result is an indication that trypanosomosis is still a problem in the study area.

Myxosporean parasites infecting Okavango catfishes

I I Rangaka[1], P H King[1] and L Basson[2]

[1]*Department of Biology, University of Limpopo, Medunsa Campus, South Africa. E-mail: pking@ul.ac.za*
[2]*Department of Zoology and Entomology, University of the Free State, Bloemfontein, South Africa. E-mail: pking@ul.ac.za*

Myxosporean parasites cause great pathology in their fish hosts. Large aggregations of plasmodium cysts in vulnerable organs such as gills and ovaries interfere with organ functions. This consequently compromises the health, reproduction and market value of fish in aquaculture industries, thus emphasising the importance of myxosporean research worldwide. The project objectives were to investigate myxosporean species invading the internal organs of 2 fish species in the Okavango River and Delta in Botswana, and to determine their taxonomic status. Fieldwork involved the collection of fish from the river using gill nets, as well as rod and line. Fish were anaesthetised with clove oil, whereafter the internal organs were removed. These were compressed between 2 glass slides and the run-off liquid was examined for live spores using light microscopy. Results revealed the presence of 6 myxosporeans belonging to 2 genera, *Myxobolus* Bütschli, 1882 and *Henneguya* Thélohan, 1892, infecting the gills and internal organs of 3 catfish species. Two *Myxobolus* spp. were found to infect the kidneys and spleen and 1 *Henneguya* sp. to infect the gills of *Clarias gariepinus,* respectively. Two *Myxobolus* spp. were found to infect the kidneys and spleen of *Clarias ngamensis*. The gills of *Schilbe intermedius* were found to harbour 1 *Henneguya* sp. Previous studies in the Okavango Delta indicated the presence of myxosporean parasites on the gills and skin of various fish species, but this is the 1st report indicating that histozoic myxosporeans also infect internal organ systems of fish in this unique ecosystem.

Identification of a small *Babesia* species found in a dog imported from Taiwan

M Troskie[1], N Mabogoane[2], O Matthee[2] and B L Penzhorn[1]

[1]*Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa. E-mail: milana.troskie@up.ac.za*
[2]*Parasites, Vectors and Vector-borne Diseases (PVVD), ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa.*

Canine babesiosis is caused by tick-transmitted intraerythrocytic protozoan parasites occurring worldwide. These were previously classified according to their morphological appearance as *Babesia canis* or *Babesia gibsoni.* Molecular studies have shown 3 vector-specific subtypes of *Babesia canis*: *B. c. rossi* (found in South Africa), B. *c. vogeli* (found worldwide), and *B. c. canis* (found in Europe). The 'small' *Babesia* parasites consist of 3 morphologically similar but genotypically distinct parasites: B*abesia gibsoni*, Asia type (found in north and eastern Africa and North America); '*Theileria*' *annae*, a parasite of dogs in Spain; and *Babesia conradae* identified in dogs in California. In South Africa, imported dogs are required to be tested for *Babesia gibsoni* by means of examination of thin Giemsa-stained blood smears and the Indirect Fluorescent Antibody (IFA) test. Recently, a blood sample from an 8-year-old Border collie cross, imported from Taiwan, was submitted. It tested positive on the IFA (reciprocal titre 1:80). The trophozoites observed on the blood smear were 'small'. DNA was extracted; the V4 variable region of the 18S rRNA gene was amplified and subjected to the reverse line blot (RLB) hybridisation assay. PCR amplicons did not hybridise with the *Babesia gibsoni*-specific probe as expected, but only with the *Babesia* genus-specific probe. Since the RLB membrane did not contain *T. annae* or *B. conradae*-specific probes, the presence of these could not be excluded. To elucidate the identity of the parasite present, the full length 18S rRNA gene will be amplified, cloned and sequenced.

Theileria-infected cell line from an African buffalo (*Syncerus caffer*)

E Zweygarth, O Koekemoer, A I Josemans, N Rambritch, R Pienaar, J Putterill, A Latif and F T Potgieter

Parasites, Vectors and Vector-borne Diseases (PVVD), ARC-Onderstepoort Veterinary Institute, Private Bag X05, Onderstepoort, 0110 South Africa. E-mail: zweygarthe@arc.agric.za

The African buffalo (*Syncerus caffer*) is a carrier of pathogenic and non-pathogenic tick-borne protozoan parasites of the genus *Theileria.* The specific diagnosis of the pathogenic species *Theileria parva* causing Corridor disease in cattle has been complicated by the presence of the non-pathogenic species in mixed infections. Identification of species-specific regions in the 18S rRNA gene has improved the specificity of diagnostic tests and allowed differentiation between certain *Theileria* species. However, there are still other hitherto uncharacterised *Theileria* species which continue to compromise the specificity of the current *T. parva*-specific real-time PCR assay in use. Therefore, the isolation and characterisation of known and novel *Theileria* species in buffalo will allow development of more accurate diagnostic tests to identify *T. parva*-infected buffalo and cattle. Here we describe the establishment of a *Theileria* sp.-infected lymphoblastoid cell line from an African buffalo in an endemic Corridor disease area in South Africa, and the partial molecular characterisation of the 18S rRNA gene of this parasite.