

# Parasitological Society of Southern Africa

The following are abstracts of papers and posters presented at the 28th Annual Congress, 20–21 October 1999, Augrabies Falls National Park, South Africa

## Abstracts of Papers

### Anthelmintic treatment of children infected by *Trichuris trichiura*

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*Trichuris* is often the most prevalent helminth in disadvantaged children in the Western Cape, and is more refractory to anthelmintic treatment than *Ascaris lumbricoides*. This makes it necessary to examine different doses of anthelmintic and treatment frequency in order to optimise the efficacy, cost benefit, management and administration of community-based intervention programmes. Accordingly, we tested 3 doses of albendazole (Zentel<sup>®</sup>, SmithKline Beecham) given at intervals of 4 months, by means of a randomised controlled trial, at Rawsonville Primary School in the Boland. Treatments were double-blind and included a placebo. The results obtained are applicable to an environment in which the geometric mean *Trichuris* egg count is approximately 1000 eggs per g of stool when quantified by the formol-ether concentration technique. Evaluation was by means of prevalence, incidence, egg reduction rate, cure rate and geometric mean egg counts. A dose of 800 mg given as a 400 mg tablet repeated the next day, appears to be optimal. A single 400 mg tablet gave inferior results, and 1200 mg gave little improvement compared to 800 mg. The need to repeat doses on 2 or 3 consecutive days to achieve total doses of 800 and 1200 mg, respectively, increases costs and will complicate compliance, management and administration.

### A preliminary investigation into the hatching conditions and host specificity of the warthog louse *Haematopinus phacochoeri*

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The warthog is the main carrier of African swine fever (ASF), a highly contagious disease fatal to most domestic pigs. For that reason transportation of live warthogs, carcasses or warthog meat products out of the ASF control area has been prohibited by Veterinary Services. Although *Ornithodoros moubata* is the invertebrate host and vector for ASF virus, the blood-sucking warthog louse may be of interest as a possible agent of mechanical transmission. The present study attempted to increase understanding of the conditions under which the nits will survive and hatch, resulting in potential infestation of domestic pigs.

Hairs laden with nits were cut from the head and skin of an infested warthog, and exposed experimentally to different ambient conditions. In an attempt to infest a domestic piglet, tufts of warthog hair laden with nits were glued to the hairs of its back. Similarly, hairs laden with nits, were placed in a bag glued to the back of a laboratory rabbit. Larvae hatched in highest numbers in a 75 % humidity chamber at  $\pm 25$  °C. No hatching was observed after the 10th day. The warthog louse seemed to be relatively host-specific, as attempts to infest a piglet and a rabbit were unsuccessful. Periodic intense grooming was, however, observed in the piglet.

### Morphology of the common snake mite *Ophionyssus natricus*

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*Ophionyssus natricus* is a cosmopolitan species that causes severe

anaemia in snakes. In heavy infestations death frequently occurs. The morphology of *O. natricus* was studied using light microscopy and scanning electron microscopy. *O. natricus* is identified by species-specific anatomical structures present on the dorsal and ventral surfaces of its idiosoma. Ventrally, the sternal plate bears 2 pairs of setae. One pair of setae is present on the genital plate and on the anal plate, 3 setae. The dorsal surface contains 2 plates, the anterior plate being larger than the posterior plate. The snake mite has more than 10 sensory setae, of varying lengths, on the tarsi of the 1st pair of legs. These legs are held up in the air when the mite is not attached to a host and it is presumed they are used as photo-receptors. Respiration occurs through stigmata situated adjacent to the 4th pair of coxae with peritreme extending past the 2nd and 3rd pairs of coxae. *O. natricus* is not common in natural populations and there is some doubt whether this mite is an obligate parasite of reptiles.

### Acaricides and *Boophilus* spp. resistance in South Africa

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Results obtained from tick larval immersion tests conducted at the South African Bureau of Standards and from a national survey on 1-host tick resistance in South Africa are confirming the increase of tick resistance against acaricides in the country. The respective objectives of the 2 surveys are to determine *Boophilus* spp. resistance against selected acaricides on farms with reported tick control problems and to determine the extent and distribution of *Boophilus* spp. resistance in South Africa against selected acaricides as indicated by larval immersion tests. The surveys involve the collection and incubation of engorged *Boophilus* spp. female ticks from animals on farms with either reported tick control problems or at randomly selected farms. The hatched larvae produced by the incubated ticks are tested, using the Shaw Larval Immersion Test, against acaricides containing active ingredients representing the synthetic pyrethroid, organophosphate and amidine groups of chemicals. For the 1st survey, the acaricides are tested for efficacy at their recommended field concentrations only. The national survey, by contrast, involves determination of LC<sub>50</sub> values of field tick strains compared to LC<sub>50</sub> values of a verified susceptible reference strain in order to calculate a resistance factor. Mortality dose data are subjected to probit analysis using the BMDP statistical package. Results obtained during the period 1996–1999 with the synthetic pyrethroid acaricides used at recommended field concentrations indicated an increase in incidence in the 0–50 % larval mortality range, while the organophosphate and amidine acaricides did not show a significant change. Only the organophosphate acaricides indicated an increase in incidence in the 51–80 % larval mortality range. The national survey results obtained to date indicate factual pyrethroid resistance, a lower incidence of organophosphate resistance and only a few cases of amidine resistance. Both surveys suggest a significant increase in the incidence of synthetic pyrethroid resistance and to a lesser extent organophosphate and amidine resistance.

### A descriptive illustration of a *Schistobranchia* species (Copepoda: Lernaepodidae) from gill filaments of the diamond ray (*Gymnura natalensis*) from South Africa

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Morphological features of a *Schistobranchia* species, collected from gill filaments of the diamond ray (butterfly ray), are presented. A single host (*Gymnura natalensis*) was caught in the shark nets of the

Natal Sharks Board, off the Durban coast, KwaZulu-Natal, South Africa. The collected copepods were fixed and preserved in 70 % ethanol. In the laboratory they were studied with the aid of bright-field and phase-contrast microscopy using the wooden slide technique. Before dissection, copepods were cleared in lactic acid into which a pinch of lignin pink had been dissolved. An examination of the morphological features of the *Schistobranchia* species revealed 2 unique characteristics that distinguish it from its congeners: firstly, the tips of its 2nd maxillae bifurcate sequentially 4 times to form a symmetrical root-like anchor and secondly, it possesses a neck-like region between the maxillipeds and the 2nd maxillae.

### Diversity of tissue changes due to protozoan and myxosporean infections in fish

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Significant advances made over the past few decades in finfish aquaculture have resulted in a wide appreciation of the potential significance of disease problems and stressed the need to recognise, prevent and control such problems in practice. The study of protozoan and myxosporean infections in wild and farmed freshwater and marine fishes concentrated on the histological changes in organs and tissues resulted in an understanding of the pathogenicity of more than 70 species, representing the phyla Rhizopoda, Mastigophora, Apicomplexa, Microspora, Ciliophora and Myxozoa. New data on the pathogenicity of well-known, common species were recognised, as well as new types of reactions of host cells and tissues. Among them are contact hypertrophy of endothelial cells, an exceptionally intensive hyperplasia of renal tubule epithelium, and xenoma transformation of the host cell in myxosporean infection. Histopathology contributed also to the knowledge of life cycles of many protozoan and myxosporean agents. The wide variety of hitherto known tissue injuries caused by myxosporeans has increased by new data on alterations of capillary complexes in the eye and swimbladder and on changes in specific sites of nervous and urinary systems. Newly-recognised alterations by extrasporogonic life cycles stages changed the existing concept of pathogenicity of some coelozoic myxosporeans. Free-living amoebae were recognised as agents of systemic infections in freshwater fishes. Pathogenetic potential of amoebae in the development of proliferative gill disease was proved by the histopathological study of gill lesions. The data provided evidence that the extent of damage to the host was defined by the species of infectious agent, its intensity and the host reaction, which in fishes constitutes a highly variable element of histopathology.

### Changes in the metabolic activities of rabbit skin at the feeding sites of *Rhipicephalus appendiculatus*

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Foreign proteins injected into the host skin at the feeding site and physical destruction of host tissue during feeding, trigger several host defence mechanisms and increased metabolic activity. An understanding of the metabolic changes at the feeding site is needed to explain differences in the host immune response when working with host-tick relationships. Little is known about this aspect of our economically-important hard ticks. It is the aim of this project to investigate metabolic changes in the skin of rabbits infested with *Rhipicephalus appendiculatus* before feeding (base-line), during early feeding and during full repletion. Histochemical procedures, including tests for fats, carbohydrates, lytic enzymes and diaphorases, were used. In unaffected skin the main area with distinct metabolic activity was the sebaceous gland tissue. Histochemical experiments using these samples and those taken 1 day after attachment, revealed no marked differences. However, marked widespread increases in host-skin metabolic activity were evident in almost all skin samples taken at the repletion stage (all host tissues demonstrated distinct fat deposition, increased diaphorase presence and clear lytic activity).

### The effect of mebendazole on the stool parasite load in children

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Recent literature indicates that more than a billion people worldwide are believed to be infected with *Ascaris lumbricoides*, 500 million with *Trichuris trichiura*, 900 million with hookworm and more than 200 million with *Schistosomes*. Although there are local and regional variations, 2 generalisations can be made; the 1st is that prevalence of infection peaks in childhood, and the 2nd that polyparasitism is common. Parasitic diseases are frequently associated with poverty and have an effect on nutrition, growth and cognitive processes in children. The aim of the current study was to monitor the effect of treatment with Mebendazole, a broad-spectrum anthelmintic marketed as Vermox (Janssen-Cilag), on children at the Othandweni Home, which is a residential care facility for abandoned black children south of Durban. The survey was carried out between October and December 1998. One-hundred-and-three children, ranging in age from 1 month to 10 years, were initially entered into the study and 65 children were assessed after treatment. Faecal samples were collected before treatment, processed in the laboratory by the formol-ether concentration method and fixed with 10 % formol saline. The concentration deposit was examined microscopically for parasite ova/cysts and expressed semi-quantitatively. Children over the age of 2 years were treated with a single 500 mg dose of Mebendazole and children under 2 years of age with 600 mg (30 ml suspension) over 3 consecutive days. A 6-week post-treatment analysis indicated that there was a 26.15 % reduction in infection in helminths (*Trichuris trichiura*, *Ascaris lumbricoides*, *Hymenolepis nana*) and 38.46 % reduction in infection in protozoa (*Entamoeba coli*; *Entamoeba histolytica*/*E. dispar*, *E. hartmanni*, *E. nana*, *Iodamoeba butschlii*, *Chilomastrix mesnili*, *Giardia lamblia*). In this preliminary study Mebendazole proved to be more effective in reducing the protozoa than the helminths, and could be of considerable value in the treatment of protozoa.

### Will reversal of helminth-induced eosinophilia enhance resistance to HIV infection and tuberculosis, slow disease progression, and improve vaccine efficacy?

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The living conditions of large numbers of people in Africa, Asia and Latin America favour infection by intestinal helminths and protozoa. Chronic helminthiasis is the main cause of eosinophilia in developing countries. Eosinophilia reflects immunological activation of Th2 lymphocytes and their cytokines. This cellular and molecular milieu is more suitable for infection by HIV and pathogenic mycobacteria than a Th1 profile. During chronic activation leading to Th2 proliferation, the rate of progression to AIDS and of tuberculosis increases and the efficacy of vaccines is likely to be impaired. A recent randomised controlled trial showed that immune-based therapy of HIV cases by injection of a Th1 cytokine restored immune function substantially. We report the results of 2 non-selective anthelmintic treatment programmes in terms of eosinophil counts. There was significant and sustained reduction of eosinophilia in the 2 groups of children. This implies switching of the immune system towards Th1. If this method can contribute to predominance of a Th1 profile on a community basis, it has the potential to reduce the incidence of HIV infection and tuberculosis, slow disease progression, enhance vaccine efficacy and confer other benefits.

## Observations on the external micromorphology of the ear mite *Otodectes cynotis* (Acari: Psoroptidae)

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*Otodectes cynotis* is a common parasite that causes ear mange in domestic and wild carnivores throughout the world. This is the 1st study to investigate the micromorphological specialisations of *O. cynotis* by scanning electron microscopy. Live mites collected from crusts taken from the ears of infested cats were killed in hot water before fixing in 70 % ethanol. After ultrasonication, the samples were routinely processed before viewing in a Leica Stereoscan 420 scanning electron microscope at 5 to 7 kV. The taxonomically-important structures, including the sexually dimorphic ambulacral structures on the legs III and IV of the adults, opisthosomal lobes and setae, as well as the precopulatory attachment organs, were examined first. These conformed to previous descriptions of *O. cynotis*. The broad dentate chelicerae enable the mites to abrade the integument of the host and ingest the epidermal debris, while any resulting exudate may be taken up by the ventral sutorial pseudorutella. The female protonymphs and tritonymphs each had a pair of prominent cup-shaped posterior copulatory tubercles to which the males attach by means of their specialised adanal suckers. The genital openings of gravid females as well as the everted aedeagus of the male mites were also examined.

## Does provision of reticulated clean water and water-borne sewage eliminate *Entamoeba histolytica*? A study of two communities in the Western Cape

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Approximately 10 % of the world's population is infected with the species-complex members *Entamoeba histolytica* and *E. dispar*, resulting in up to 5 million cases of invasive amoebiasis and 100 000 deaths annually. Projected to South Africa, these figures could be 45 000 cases of invasive amoebiasis per annum with 1000 deaths. It is often stated that provision of water-borne sewage and good-quality reticulated water is the most effective means of controlling transmission of *Entamoeba* and thereby preventing invasive amoebiasis in a community. Two communities were studied in the Cape, a region of South Africa considered by many to be a low-risk area for amoebiasis. The 1st comprised 290 subjects (mainly children) living in formal accommodation at Langebaan on the West Coast with water-borne sewage and reticulated clean water; all subjects in the 2nd community were rural children living on wine farms with minimal sanitation around Rawsonville in the Boland. Stools and blood were collected and it was possible to determine the prevalence of *E. histolytica* and *E. dispar* using a combination of microscopy, serology and zymodeme analysis. Although subjects in both communities were found to harbour non-pathogenic *E. dispar* infections, pathogenic *E. histolytica* infections were only found in approximately 2 % of the children in Langebaan. The implications and consequences of transmission of pathogenic *E. histolytica* in a community apparently enjoying a high level of sanitation is disturbing. As this is contrary to currently accepted beliefs, transmission pathways need to be determined. Epidemiological analysis of the data is being undertaken at present and this could provide clues regarding parasite transmission in these 2 areas.

## Freshwater snails and cercarial shedding in the North West Province

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Freshwater snails are known to serve as hosts for a variety of parasites of medical and veterinary importance. Snails were

sampled in an area 100 km northwest of MEDUNSA and examined for cercarial shedding. Snails were identified and put out in glass containers to observe cercarial shedding. The cercariae were collected, identified and studied using a light- and scanning electron microscope. Three snail species were sampled and between them they shed 7 different types of cercariae. *Bulinus africanus* shed an apharyngeal brevifurcate distome fork-tail cercaria that was experimentally tested and identified as *Schistosoma mattheei*. *Bulinus tropicus* shed 5 types of cercariae. Two of them are echinostome cercariae with their collars housing 27 and 45 spines, respectively. It also shed a xiphidio cercaria with its typical xiphidio-spine at the anterior end of the body. A fork-tail cercaria was identified as a pharyngeal longifurcate distome cercaria. It also shed an amphistome cercaria that results in the parasite *Calicophoron microbothrium* in cattle and sheep. *Lymnaea natalensis* shed an apharyngeal brevifurcate distome cercaria. Of the above-mentioned cercariae, 2 are responsible for well-known parasites in cattle, but the remaining 5 are unknown and their effect in the community is not known. Follow-up studies are therefore necessary.

## Determination of viability of *Giardia lamblia* cysts: vital staining, *in vitro* and *in vivo* excystation

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*Giardia lamblia* infects a large number of people worldwide. Apart from having peak prevalence in children between 1 and 5 years owing to lack of personal hygiene, one of the modes of transmission is *via* contaminated water supplies. This results in widespread transmission and waterborne epidemics of giardiasis. For 6 years (1981–1986), giardiasis was consistently documented as one of the most frequently occurring waterborne diseases transmitted by drinking water in the United States, being responsible for a total of 53 outbreaks during that period. It is therefore important for water-purifying systems to reliably determine the efficacy of methods employed in the purification systems. Detection of viable *Giardia* cysts from water sources is one of the methods of assessing the efficacy of purifying agents. In this study, different methods of determining cyst viability were assessed. Fifty-two batches of fresh cysts were obtained from faeces collected from hospital patients and presumably healthy institutionalised children. Cysts were separated on 1M sucrose gradients and viability assessed by (a) vital staining using 0.01 % eosin, (b) excystation *in vitro* using acid pepsin and (c) *in vivo* excystation by inoculation of neonatal mice. In all 52 samples analysed by vital staining, some level of viability was detected, the percentage viability ranged between 20 and 100 %. Nineteen of the 52 cyst samples excysted *in vitro* (37 %) while 43 % excysted in mouse intestines as evidenced by the presence of trophozoites in sacrificed mice. Ten specimens were excysted using both methods. Eight excysted *in vitro* only while 10 excysted *in vivo* only. The implications of these results in terms of the value of the methods employed in this study in determining *Giardia* cyst viability is discussed.

## Extensive morphological changes in the adult female *Afrolernaea mormyroides* Van As, 1983, during metamorphosis

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*Afrolernaea* Fryer, 1956, is 1 of 13 genera belonging to the family Lernaedidae. It is accepted that this small group of fish ectoparasites has reached the highest degree of adaptation to parasitism among the cyclopoid copepods. *Afrolernaea* is endemic to Africa and mainly associated with mormyrid fishes. During seasonal surveys at the Nwanedi-Luphephe Dams in the Northern Province, some developmental stages (pre-metamorphosis female and adult female) were collected from the host, *Marcusenius macrolepidotus*. After removal from the hosts, parasites were fixed and stored in 70 % ethanol. Specimens selected for scanning electron microscopy were carefully cleaned and dehydrated to absolute ethanol. After

dehydration, specimens were critical-point dried and sputter-coated with gold. Specimens were viewed and micrographed in a Jeol 610 Scanning Electron Microscope at 4 kV. Development of *Afroloernaea* involves several free-living naupliar and copepodid stages. The pre-metamorphosis female is confronted with a great challenge, the need to enter into a close and intimate relationship with the host, resulting in extensive morphological changes. Some of these changes include: great extension in body length (development of a neck); almost complete loss of external segmentation; apparent complete loss of antennules and antennae; expansion of the genital region; modification of cephalic appendages to develop into hold-fast structures (maxillae and maxillipeds); enlargement, sclerotisation and transformation of 1st thoracic legs into attachment hooks; the transformation of remaining thoracic legs and furcal rami into rudimentary appendages. A morphological study was undertaken and the appendages of the last copepodid/pre-metamorphosis female were compared with that of the adult female. Special reference is made to thoracic legs 2–5, not previously reported for *A. mormyroides*. The possible reasons for these morphological changes are addressed.

### Occurrence and diversity of bovine coccidia at three localities in South Africa

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Information on the occurrence and diversity of coccidia affecting cattle in different farming systems in South Africa is lacking. The objective of the study was to determine the levels of infection of coccidia in cattle at 3 localities and to determine the *Eimeria* species occurring at each locality. A total of 1936 faecal specimens were collected over a 13-month period from a dairy farm (Malleasons), a Bonsmara breeding farm (Kaalplaas) and a Nguni stud farm (Pienaars River). Samples were collected from adults >24 months and calves <6 months old. The modified McMaster technique was used to determine OPG (oocysts per gram of faeces). Samples with OPGs >2000 were allowed to sporulate in potassium dichromate solution at room temperature. Species identification was performed by measurement and morphological characteristics of each species. At Malleasons, 28 % of specimens were positive, Kaalplaas 52 % and Pienaars River 49 %. The most prevalent species were *E. zuernii* and *E. ellipsoidalis* at Malleasons, *E. zuernii* and *E. bovis* at Kaalplaas and *E. bovis* and *E. zuernii* at Pienaars River, respectively. Adults had very low OPGs. There was a pattern at the 3 localities in that high OPGs were only recorded in calves, which were also shedding the most pathogenic species.

### SEM observations and prevalence of *Microcotyle van Beneden* and *Hesse*, 1863 (Monogenea: Microcotylidae), infections in wild and cultured spotted grunter, *Pomadasys commersonii*

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Diseases caused by helminths are more frequent in captive fishes (research cultivation and commercial cultivation) than in their free-living counterparts. Interestingly, cultured fish lose many of their helminths, while protozoans and copepods affect them in increased numbers. The spotted grunter was identified by the mariculture thrust for future commercial cultivation and the study of parasites of this species was initiated. The focus is on the only monogenean retrieved from the gills of both wild ( $n = 125$ ) and cultured ( $n = 67$ ) fish over a period of 8 seasons. Fish samples caught by seine and gill nets were grouped according to total length. A subsample was examined immediately for parasites and another was held captive in tanks to be examined at least 1 season later. Parasites were fixed in 70 % ethanol. For SEM, specimens were dehydrated in graded ethanol, critical-point dried with CO<sub>2</sub> in absolute ethanol, sputter-coated in gold and micrographed with a JEOL 6100 at 5 kV. The taxonomically important features are

described. Parasites were also retrieved from fry recently recruited to the estuary, suggesting that infections arise during early life of the fish. Only 1 cultured specimen was infected. The use of 2 phenoxy-ethanol (anaesthetic) during routine monitoring contributed to the disappearance of the parasites.

### Evaluation of a standardised direct agglutination test for the diagnosis of visceral leishmaniasis in Kenya

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A World Health Organization (WHO) Direct Agglutination Test (DAT) prototype test kit was evaluated in Baringo District, Rift Valley Province, Kenya. Approximately 10 000 individuals were screened for visceral leishmaniasis (VL), and of these, 609 filter paper samples from 305 VL clinical suspects matched for sex and age ( $\pm 2$  years) with 304 controls were screened using this kit. A DAT titre of  $\geq 1:2000$  was considered positive for VL. The screening process was done in 3 stages. The 1st stage (field screening) involved screening of all filter papers from both suspects and controls at a DAT titre of 1:500. The 2nd stage (laboratory titration) involved screening of the same individuals but using freshly-eluted filter paper samples with a view to rescreening all blood samples at 1:500 to 1:2000 dilution. For the 3rd stage (full-scale titration) all samples that were positive at 1:2000 qualified for a full-scale titration of 1:500–1:512 000. All the DAT-positive ( $\geq 1:2000$ ) VL-suspects were further subjected to a confirmatory splenic aspirate by both smear and culture. Those found to be parasitologically positive were put on a standard treatment regime of 20 mg/kg/day of sodium stibogluconate (Pentostam<sup>®</sup>). Of the 305 VL clinical suspects screened, 42 were DAT-positive ( $\geq 1:2000$ ), giving a prevalence of VL among suspect cases as 42/305 (13.8 %). Of the 42 DAT-positive ( $\geq 1:2000$ ) cases, only 32 were further diagnosed by splenic aspirate. All the 28 parasitologically confirmed VL patients were positive by DAT ( $\geq 1:2000$ ) while 4/32 (12.5 %) DAT-positive ( $\geq 1:2000$ ) cases were false positives. At 3 months, follow-up of 4 serologically positive but parasitologically negative VL suspects, 1 suspect had seroconverted, while the 2nd had a lower DAT titre of 1:32 000 than the original 1:128 000, and both had no palpable spleens. All parasitologically confirmed patients were DAT-positive ( $\geq 1:2000$ ). One patient (S180) was DAT-positive (1:64 000) while negative on smear but positive on culture. The rest were all positive in both smear and culture. The kit has a low cut-off titre of 1:2000, making it suitable for use at all levels of primary health services in screening of rural communities at risk of infection, and with limited trained and skilful personnel.

### PCR for the detection and species identification of intestinal microsporidia

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Intestinal microsporidia (*E. bienersi* and *E. intestinalis*) are obligate, intracellular protozoan parasites, causing diarrhoea mainly in the immunocompromised (HIV) patients. The diagnosis of intestinal microsporidiosis was initially performed by duodenal biopsy analysis, using light microscopy. Currently, the diagnosis is made by microscopic examination (modified trichrome stain and the Uvitex 2B stain – a fluorescent method) of stool samples. Although microscopy on stool samples is a highly sensitive and specific technique, conclusive species identification is not possible. Chemotherapy (using Albendazole) is successful for treating only *E. intestinalis* and not *E. bienersi*, thus making species identification of intestinal microsporidia important. Species identification is at present achieved through transmission electron microscopy (TEM), but the cost and expertise needed to sustain this technology outweighs its

benefits to a diagnostic laboratory. The polymerase chain reaction is employed in this study to amplify and detect microsporidial DNA in stool samples. Differentiation to species level uses a restriction enzyme (*Pst*I). Various extraction techniques were evaluated to determine which technique was most efficient in extracting microsporidial DNA. A simple 'boiling extraction' (BE) technique and a chemical extraction technique – the 'Dithiothreitol' (DT) method gave good results when tested on known positive samples. They were thus used in a controlled experiment to ascertain their sensitivity and specificity. A primer pair described in the literature amplified microsporidial DNA from all species of microsporidia, and the products were examined by agarose gel electrophoresis. A study was designed to compare the 2 extraction techniques (BE, DT) using 30 samples (20 negative and 10 positive) by microscopy. The BE extraction technique gave 80 % sensitivity and 100 % specificity similar to the DT method which gave 70 % sensitivity and 100 % specificity for detecting microsporidial DNA. All the positive amplicons were not digested by (*Pst*I) one restriction enzyme, confirming *E. bieneusi*. The microsporidial positive samples (microscopy) were examined by TEM and the results correlated 100 % with that of the restriction endonuclease analysis (*i.e.* all the test samples were *E. bieneusi*). The PCR method was able to detect 3 spores in 2.5 µl of stool sample. The BE method is a simple, cost-effective way to extract microsporidial DNA. It was able to reduce extraction time from 24 hours to 30 minutes.

### Blackflies in South Africa – their control and the fear of resistance

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Blackflies are notorious pests along almost all the major rivers in South Africa. The problem is caused by the blood-feeding females that need protein for egg development and are therefore compelled to feed on humans, livestock, poultry, birds and wildlife. Along the Orange River alone it is estimated that blackflies are capable of causing stock losses amounting to R88 million per annum. Initially the blackfly problem in South Africa was confined to the Vaal River, but with the expansion of irrigation schemes and hydroelectricity, blackflies have also spread to the Orange, Great Fish and Sundays Rivers. Periodic outbreaks are also reported from the Gamtoos, Eerste and Berg Rivers. The Vaal River problem is caused by *Simulium damnosum*, and was initially controlled by DDT applications, but owing to the environmental damage caused by the larvicide, water-flow manipulation was later used. Water-flow manipulation was also used against *Simulium chutteri* in the Orange River, but with the expansion of irrigation along the Orange River this method became impractical. The ARC - Onderstepoort Veterinary Institute therefore started experimenting with larvicides. This led to the registration of *Bacillus thuringiensis* var. *israelensis* De Barjac (*Bti*) and the organophosphate, temephos, as larvicides for blackfly control in South Africa. Although both these larvicides are safe to use in South African rivers, *Bti* is preferred owing to its lower environmental impact. However, *Bti* is ineffective during periods of high flow (>150 m<sup>3</sup>/s) and periods of high algal (>1500 cells/ml) and total suspended solids (>150 mg/l) concentrations, so during these periods temephos must be used. From 1992–1995 the river flow in the Orange River was in general very low and *Bti* was used often and temephos only periodically. However, from 1996–1998 periods of sustained high flow were experienced, and this led to repeated temephos applications. This alerted us to the possibility of temephos resistance, as blackfly resistance to temephos in other parts of the world is well documented. Should blackflies become resistant to temephos, the Orange River blackfly control programme will be in serious danger of being ineffective. It is therefore concluded that temephos and *Bti* must be used rotationally with preference given to *Bti*, and that temephos resistance should be monitored carefully. Researchers should also look for a 3rd alternative larvicide belonging to a different insecticide group. Blackfly resistance to *Bti* has never been reported, and it can thus be used with confidence as the only larvicide in the smaller rivers like the Great Fish, Sundays and Gamtoos.

### *Kroyeria* sp. (Kroyeriidae: Siphonostomatoida), a parasitic copepod infesting gills of spinner sharks (*Carcharhinus brevipinna* (Müller & Henle, 1838)) in the Indian Ocean

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*Kroyeria* sp. (Kroyeriidae: Siphonostomatoida) is described from male and female specimens collected from gills of spinner sharks (*Carcharhinus brevipinna* (Müller & Henle, 1838)) captured in the Indian Ocean, off the coast of South Africa. The collected copepods were fixed and preserved in 70 % ethanol. In the laboratory they were studied with the aid of brightfield and phase-contrast microscopy using the wooden slide technique. Before dissection, copepods were cleared in lactic acid into which a pinch of lignin pink had been dissolved. This species can easily be distinguished from other *Kroyeria* species by the length of the last endopod segment and armature of the swimming legs.

### Transplacental transmission of *Babesia* (= *Theileria*?) *equi* in horses: the rule rather than the exception?

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Abortion due to *Babesia equi*, a major problem in Thoroughbred breeding in South Africa, is assumed to occur whenever the parasite crosses the placenta. We attempted to ascertain whether carrier mares could give birth to infected but clinically normal foals. A newly-developed DNA probe was used to confirm the presence of *B. equi* parasites in the spleens of 6 foetuses that were mechanically aborted from known parasite-carrier mares between 4 and 7 months of gestation, and in blood samples collected from 7 healthy new-born foals of known carrier mares. The progress of congenital *B. equi* infections was followed in 3 foals born in a tick-free facility and kept there with their dams for 2–3 weeks. The probe detected *B. equi* in all 6 foetuses and in all 7 new-born foals. The probe further detected *B. equi* in the blood of the 3 foals from birth to termination of the trial, thus demonstrating the persistence of congenital infections, at least to 3 weeks *post partum*, even in the presence of colostral antibodies. We conclude that transplacental transmission of *B. equi* in carrier mares is the rule rather than the exception, and that it probably takes place during the first 4 months of gestation. Unfortunately, we are no closer to understanding the processes that may lead to abortion.

### Anthelmintic resistance: conventional recommendations for control reviewed in relation to novel methods of biological worm control

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Anthelmintic resistance in *Haemonchus contortus* has already progressed so far that some farmers now have only a single compound left with apparently undiminished efficacy. Because *H. contortus*, the predominant species in sheep and goats in the entire summer rainfall region of South Africa, is lethal, it is not economically feasible to farm with small stock unless effective anthelmintics are available. Thus, resistance seriously threatens sustainable small-stock production. The rampant anthelmintic resistance indicates incorrect application of the anthelmintics, necessitating re-evaluation of conventional worm control recommendations for the potential to select for resistance, particularly in the light of some novel methods of biological worm control: 1) the FAMACHA<sup>®</sup> system of clinical evaluation of anaemia resulting from haematophagous worm infection makes it practicable to drench only those individual sheep and goats that are in danger of succumbing to the worm challenge; 2) obtaining reversion of resistance to susceptibility, by replacing or greatly diluting resistant with susceptible worm strains; 3) the '50–50'

grazing system, that comprises annually resting half of the available pasture for practically a full year, to maintain sustainable pasture production. A succession of reviews have pointed to the potential of the conventional recommendations to select for anthelmintic resistance, but there appears to have been a reluctance: 1) to condemn those techniques; 2) to select the best alternatives, and 3) to find ways of making a greater input for less effective worm control acceptable. What is required is not only to point out the potential hazard of continued use of the existing systems, but also to set up an alternative for each one, and to start the long process of re-educating the farmers and their advisers. In South Africa we are already finding that those individual sheep farmers who are faced with the very real prospect of having no more effective anthelmintics with which to control *H. contortus*, are much more prepared than the 'average' farmer, to apply the more exacting and unpopular methods of integrated worm control. In particular the following universal recommendations are critically evaluated: using grazing management to provide 'low worm' pastures; timing strategic drenches to reduce contamination of pastures with worm eggs; avoiding under-drenching; and alternation of anthelmintics.

### Modifications made to the parasite index developed as water quality monitoring tool in the Olifants River

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During the 1998 conference the development of a parasite index was discussed, and the current paper deals with refinements made to the original hypothesis. Originally it was stated that the more ectoparasites present, the better the water quality of the river or dam being tested. However, analysis of data collected from *Clarias gariepinus*, at 2 river points in the lower Olifants River catchment area, indicated that the presence of endoparasites, white blood cell count and blood haematocrit values provided a significant statistical difference between sample sites. A significance value of 81.08 % was obtained when a logistic regression model was employed. Furthermore, a significant difference of 82.35 % was obtained when data from 2 dams in the upper Olifants River catchment area were statistically analysed by a logistic regression model selected for blood plasma protein levels, presence of endoparasites and condition of the spleen. Ectoparasites were excluded from the model in both cases, as this variable was seen to provide no significant difference between the sample sites. The use of gill nets as a capture technique may well be responsible for the loss of ectoparasites before they can be counted.

### Abstracts of Posters

#### *In vitro* antimalarial activity of selected South African medicinal plants

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With the widespread emergence of resistance of *Plasmodium falciparum* to many classic antimalarial agents, attention has turned to the possibility of deriving novel antimalarial drugs from plants. It is estimated that 80 % of the people living in developing countries, including South Africa, almost exclusively use traditional plant medicines for health care. Thus, the aim of this study was to investigate plants used by South African traditional healers for antimalarial activity against chloroquine-resistant *P. falciparum* (FCR-3). Seven plant species were collected, air-dried and finely ground. Cold and hot aqueous extracts were prepared and lyophilised. The extracts were solubilised, the pH corrected to 7.4 and the osmolarity adjusted to 300 mOsm. The antimalarial activity of each plant extract was determined by measuring the uptake of <sup>3</sup>H-hypoxanthine into the malaria parasites as an indicator of growth. Preliminary results show that 2 of the 7 plants have promising antimalarial activity, with the 50 % inhibitory concentration being below 300 µg/ml. These initial data indicate that these plants warrant further investigation

into their antimalarial activity and toxicity profiles.

### The occurrence of pollution-induced epitheliocystis-like damage to the gills of *Clarias gariepinus*

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Chlamydial organisms are suspected of causing epitheliocystis. The condition is characterised by hypertrophy of integumental epithelial cells. Infected cells are visible as small, white, cyst-like excrescences on the gills of affected fish. During this study, catfish (*Clarias gariepinus*) were collected from the Vaal Dam and Vaal River Barrage bi-monthly with gill nets. Gills were dissected out and examined for hypertrophy and hyperplasia using a dissection microscope. Unlike the Vaal Dam, the Barrage receives industrial and domestic waste effluent from the Vanderbijlpark-Vereeniging complex. This is reflected by the poorer water quality at this site. Prevalence and intensity of gill damage were calculated for all seasons. Both were higher at the more polluted locality, with prevalence differing as much as 54 % between localities in autumn. It is concluded that pollution increases the prevalence and intensity of gill damage resembling epitheliocystis infection. No histological examinations of the affected tissue were performed. Conclusions regarding pollutant action on infection of gill epithelium by chlamydial bacteria should thus remain tentative.

### The mysterious *Paeon*

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*Paeon* (Siphonostomatoida, Copepoda) (*Peaon* = a son of Poseidon) was established in 1920 as a new genus of the family Sphyrriidae. All the females of *Paeon* species have very peculiar protuberances on the cephalic region totally obscuring the mouth tube and appendages. Specimens of *P. elongatus* were collected from dusky sharks, *Carcharhinus obscurus*, caught in the shark nets of the Natal Sharks Board, off the KwaZulu-Natal coast. Material was fixed and preserved in 70 % ethanol, and studied with the aid of brightfield and phase-contrast microscopy using the wooden slide technique. Before dissection, specimens were cleared in lactic acid into which a pinch of lignin pink had been dissolved. Morphological features of *P. elongatus* were also examined using scanning electron microscopy (SEM). These micrographs are used to help solve the mystery surrounding the cephalic appendages. After the removal of the anterior pair of protuberances, the 1st antennae, 2nd antennae, 1st maxillae and maxillipeds could be observed.

#### *In vitro* screening of traditionally used medicinal plants for antimalarial activity

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The emergence of drug-resistant strains of malaria indicates the need for research to find new chemotherapeutic agents for the treatment and prophylaxis of malaria. South Africa has a diverse plant kingdom that is widely used by traditional healers. This warrants scientific research to investigate the efficacy and safety of these plants. Four plant species from the genera of *Crinum*, *Eucomis*, *Gunnera* and *Pentanisa* that are used locally in traditional herbal medicine were tested for antimalarial activity against the chloroquine-resistant strain *Plasmodium falciparum* (FCR-3). Harvested plant material was air-dried and milled. Extractions were performed using hexane, methanol and water. Organic solvents were evaporated off while the water extracts were lyophilised. The plant water extracts were adjusted to physiological pH and the osmolarity was adjusted to 300 mOsm. Using the <sup>3</sup>H-hypoxanthine incorporation assay as an indicator of parasite growth the IC<sub>50</sub> values for the water extracts were determined over a single cycle (48 h). The preliminary

screening of the hot- and cold-water extracts indicate the presence of antimalarial activity against *P. falciparum* (FCR-3) *in vitro*. The IC<sub>50</sub> values obtained range from 2 µg/ml to 1000 µg/ml with little variation occurring between the hot and cold aqueous extracts. Plant species with IC<sub>50</sub> values below 10 µg/ml warrant further investigation to determine not only their antimalarial activity but also the potential toxicity of the extracts.

### SEM observations on the micromorphology of the warthog louse (*Haematopinus phacochoeri*)

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*Haematopinus phacochoeri* is a common ectoparasite of the warthog, which is the main host of African swine fever (ASF), a virulent disease with a high mortality rate among domestic pigs. This study investigated the micromorphological specialisations of *H. phacochoeri*. Live lice were collected from an infested warthog and fixed directly in 70 % ethanol. Specimens were routinely processed for scanning electron microscopy and viewed in a Leica Stereoscan 420 at 5 to 7 kV. This robust louse (6 mm in length) has a fused thorax characterised dorsally by 2 notal pits on the prothorax, a pair of large spiracles and a large central notal pit on the mesothorax, while 2 pointed processes extended posteriorly from the metathorax. The membranous abdomen was protected dorsally by rows of sclerotised tergites, and laterally by large bulbous double paratergites on segments 3 to 8, each containing a tubular spiracle. Each leg had a large claw for gripping the hair against its specialised tibial thumb and unique distotibial pad, thus forming 6 powerful 'grasping organs' for attachment. The tubular haustellum ensheathed the 6 piercing stylets on the anterior tip of the cone-shaped head with its prominent ocular points. The antennal sensoria consisted of a terminal peg organ with 12 sensilla, 2 pore organs, as well as a double plate organ for the orientation of this eyeless louse.

### Morphology of a *Paradiplozoon* sp. from *Barbus aeneus* in the Vaal River system

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In Africa only a few species of the family Diplozoidae have been described, namely *Diplozoon aegyptensis* Fischthal and Kuntz, 1963, from Egypt, Kenya and Uganda, collected from *Labeo* spp. and *Barbus* spp.; *D. ghanense* Thomas, 1957, collected from *Alestes macrolepidotus* in Ghana and *Neodiplozoon polycotyleus* Paperna, 1973, on *Labeo* spp. and *Barbus* spp. in Kenya and Tanzania. An investigation was undertaken in the Vaal Dam. Possible host fish species were collected with gill nets. The fish were killed and the length, weight, sex and approximate age were determined. The gills of the fish were removed and examined with a dissection microscope. The position of the parasites on the gill filaments was recorded. Thereafter parasites were removed and fixed between a cover slip and glass slide in warm aceto-formaldehyde alcohol and preserved in 70 % ethanol. Morphology was studied with a Zeiss standard 16 microscope as well as a JEOL 5600 scanning electron microscope. Whole mounts were prepared with boraxcarmine-iodine and haematoxylin staining methods. Serial sections of resin embedded parasites were made at 5 µm and stained with trichrome. The parasites are relatively large (±4 mm) in comparison to the gill filaments of the host fish *Barbus aeneus*. The anterior part of the parasite has 2 muscular buccal suckers and a pharynx that extends into a branched intestine. The posterior section of each hermaphrodite worm is filled by the reproductive system, and terminates in an opisthaptor equipped with 4 pairs of clamps. As the parasite has 4 pairs of clamps, a reproductive system that is situated posteriorly and the presence of an egg without a filament, the conclusion can be drawn that this organism is a representative of the *Paradiplozoon* genus. This genus has not yet been described in Africa.

### Comparative study of water relationships and fecundity in ixodid tick eggs

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The survival and hatching of eggs are critical phases in the life cycle of ticks. Little is, however, known about the water balance physiology of tick eggs. This study was designed to determine the rate of water loss and the ability of tick eggs to absorb atmospheric water. Six different ixodid tick species were used. Females were kept in separate containers at 90 % RH and 25 °C and allowed to oviposit. Eggs produced on the 3rd and 14th day were harvested. Eggs from each of these days were weighed and stored in a desiccator at 25 °C and weighed daily. After 30 % of the initial weight had been lost, the eggs were transferred back to 90 % RH and 25 °C and weighed after 2 days to determine any weight change. *Amblyomma marmoreum* eggs were the largest and lost 30 % of their initial weight within 1.4 days and the eggs were also able to absorb atmospheric water. This phenomenon was also observed for *Ixodes rubicundus*, whose eggs also displayed the lowest rate of water loss. *Hyalomma m. rufipes*, *Rhipicephalus sanguineus*, *Haemaphysalis leachi* and *Boophilus decoloratus* eggs did not have the ability to absorb water vapour after desiccation. Eggs of species with longer incubation periods tend to be more resistant to desiccation than those with shorter incubation periods.

### Technology transfer to resource-limited farmers from a helminthological point of view

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The transfer of technology and information to resource-limited farmers is one of the most difficult challenges facing the scientific community in South Africa today, as also in other developing countries. Illiteracy, language barriers, traditions, communal pasture systems and lack of infrastructure are but a few of the obstacles in the way of progress. Special aids and amended, user-friendly information material have to be invented, developed and tested for efficiency before being released for use in the field. What is exciting today, is the use of computers that are becoming available even to those who are not computer-trained. Everyone can contribute with the help of these aids to the educational needs in South Africa. Technology transfer is an important and valuable means of reaching farming communities, providing essential training that is desperately needed. Knowledge about helminth parasites ensures a safe symbiosis between humans and animals. Unless the developing farmer is aware of the pathogenic effect of particularly wireworm and brown stomach worm, they have little chance of improving the production of sheep and goats to commercially viable levels.

### The antimalarial effect of some colourants and purine derivatives on *Plasmodium falciparum*

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Several colourants and purine derivatives have been shown to have an *in vitro* inhibitory effect on cyclin-dependent kinases (CDKs). CDKs play an essential role in the control of the cell division cycle. In view of this role, CDKs have been the object of considerable investigation. Several CDK homologues have been characterised in *P. falciparum*. Several colourants and purine derivatives have been found to have an *in vitro* inhibitory effect on the isolated yeast CDK enzyme, *cdc2*. The aim of this study was to determine the effect of the following colourants, briebrich scarlet, palatine chrome black 6BN and toluidine red, and 2 purine derivatives, called 97 and 97M, on the chloroquine-resistant *P. falciparum* (FCR-3) strain. The effect of

the colourants and purine derivatives was determined by the <sup>3</sup>H-hypoxanthine incorporation assay over a double cycle. The erythrocytic stage of the chloroquine-resistant strain (FCR-3) was maintained in continuous culture and exposed to various concentrations of the colourants and purine derivatives over a double cycle (78 h). Preliminary data indicate the IC<sub>50</sub> of the colourants to be 19 μM for palatine chrome black 6BN, 20 μM for briebich scarlet and 28 μM for toluidine red. The IC<sub>50</sub>s of the purine derivatives was 3.7 μM for 97 and 0.88 μM for 97M. These data indicate that the colourants and purine derivatives warrant further investigation into their anti-malarial properties.

### The influence of temperature and humidity on the immature stages of *Ctenocephalides felis*

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Only about 1 % of a potential flea population is in the adult stage at any particular time. Because of the small size and nature of the flea in its immature stages, we only come into contact with adult fleas. The other stages of the life cycle allow an ever-continuing development of fleas even when all the adult fleas of a current generation are killed. The key, then, to control fleas, is to consistently interrupt their life cycle at an immature stage so that they do not develop into adults. Because eggs and larvae are extremely susceptible to heat and desiccation, it is necessary to determine the conditions under which eggs will remain viable and hatch, as well as the optimum conditions for larval development. Eggs and larvae, kept in Petri dishes, were exposed to different temperatures and humidities, weighed and monitored daily. Eggs hatched faster and more successfully at higher temperatures and humidities, and the larvae were more active. Although the rate of moulting between different larval instars increased at higher temperatures, the most favourable conditions, which gave the highest survival rate, were found to be moderate conditions. Survival rates varied between 40 % and 50 % at 15 °C and 75 % relative humidity compared to between 60 % and 65 % at 35 °C, but were highest, namely between 80 % and 90 %, at 25 °C and 75 % relative humidity. At temperature 25 °C and 50 % relative humidity, between 95 % and 100 % of eggs hatched within 3 days, while between 65 % and 70 % of the eggs hatched in 2–4 days at 90 % relative humidity.

### The effect of chemical colourants on *Plasmodium falciparum*

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A family of proteins called cyclin dependent kinases (CDKs) regulates the progression of the eukaryotic cell cycle at discrete points. These kinases play a vital role in the proliferation processes of the cell. Their activity is controlled by a group of proteins called cyclins. Several CDK-like homologues have already been characterised in *P. falciparum*. The *in vitro* enzyme assay on a yeast CDK (*cdc2*) shows that certain chemical colourants have an inhibitory effect on enzyme activity. Therefore, the aim of this study was to investigate the antimalarial effects of these chemical colourants on *P. falciparum* and to assess their potential toxic effects on human cells. A chloroquine resistant strain (FCR-3) of *P. falciparum* was maintained in continu-

ous culture. Using the radio-labelled hypoxanthine incorporation assay as an indicator of parasite survival, the IC<sub>50</sub> values of para red and crocein orange-G were determined over a double cycle (78 hours). Preliminary results indicate that para red and crocein orange-G have IC<sub>50</sub> values of 1.73 μM and 26.8 μM, respectively. These results warrant further investigation on para red and other structurally related compounds.

### Some parasites of the african pike, *Hepsetus odoe*, from the Okavango Delta, Botswana

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The African pike, *Hepsetus odoe*, is an endemic African freshwater fish that belongs to the monospecific family Hepsetidae within the order characiformes. This species is widely distributed throughout west and central Africa from Senegal southwards to Botswana, where the Okavango forms the southernmost limit of its distribution. During 3 extensive field trips to the Okavango Delta, October 1997, June–August 1998 and June–July 1999, more than 60 specimens of *H. odoe* were collected and examined for parasites. Collection methods included gill nets, cast nets as well as fishing rods. After collection the fishes were taken to a fully equipped field laboratory where every specimen was examined for different groups of parasites occurring in and on the host. All parasites found were removed and fixed according to standard methods for their specific group. *Hepsetus odoe* is infested with a wide variety of parasites. These include a *Trichodina* sp. from the skin and gills, a *Tripartiella* sp. from the gills, *Myxobolus africanus* from the gills, *Ichthyobodo necator* from the gills, *Lamproglena* sp. from the gills, adult nematodes in the gill arch, adult acanthocephalans from the intestine and *Annulotrema hepseti* from the gills.

### 'Malkopsiekte' – 'the forgotten danger' – a recent case study in the southern Free State

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'Malkopsiekte', also referred to as gid, sturdy or staggers, primarily occurs in sheep and is caused by the larval stage (*Coenurus cerebralis*) of *Taenia multiceps*, a tapeworm of dogs and other wild carnivores. The cysts occur in the brain and sometimes the spinal cord of the intermediate hosts, which include sheep, goats, cattle, horses and other ruminants. This disease can also infect man (zoonosis) and is potentially fatal if not treated promptly. A farmer in the southern Free State has lost between 50 and 60 sheep every year for the past 4 years apparently due to *T. multiceps*. These animals show signs of emaciation, loss of appetite and apparent intermittent blindness. The affected animals were inclined to wander off aimlessly, head held low and depressed. Six sheep were taken to the Faculty of Veterinary Science for necropsy and *T. multiceps* was diagnosed.