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Possible dissemination of eggs of whipworm (*Trichuris trichiura*) and roundworm (*Ascaris lumbricoides*) by wind in the Western Cape

V J Arendse[a], J M G van Deventer[b], J Fincham[a], J Austin[a] and A C Evans[a]
[a]*National Research Program for Nutritional Intervention, Medical Research Council, PO Box 19070, Tygerberg, 7505 South Africa.*
[b]*Parasitology Research Programme, University of the Witwatersrand, Johannesburg, South Africa.*

In a West Coast community with basic municipal services, the prevalence of *Trichuris* infection in primary school children was 72 % ($n = 307$). In a Boland farming community, it was 78 % ($n = 496$), and in a community in transition on the Cape Flats, 84.2 % ($n = 316$). Sand particles were frequently noted during microscopy of stool. In Tokyo, *Ascaris* eggs were found in dust in homes, classrooms and buses. Persistent strong wind is characteristic of the Western Cape climate, and its possible role in disseminating worm eggs should be explored. Eggs were present in dust collected from air conditioners in a building. A dust trap consisting of a metal tube with a fine gauze (20 Fm) on the inside acting as a sieve, was designed and placed outside. The design of the trap allows it to turn into the direction of the prevailing wind. Preliminary results were positive. A more efficient dust trap is being designed to quantify the number of eggs per volume of air. Seasonal effects, if any, will also be investigated, as well as the viability of eggs trapped. Faeces of rodents and birds are being examined for eggs similar to those of *Trichuris trichiura.* On account of its relative resistance to anthelmintics, and widespread occurrence, the epidemiology of whipworm must be defined.

Morphological anomalies in *Mugilicola* spp.

A Avenant-Oldewage
Department of Zoology, Rand Afrikaans University, PO Box 524, Auckland Park, 2006 South Africa.

Four species of *Mugilicola* have been described from India, Iraq, Australia and South Africa; only *Mugilicola smithae* occurs in South Africa. The type specimens were collected from eels in rivers in the Transkei. This species differs from the remainder in that it possesses a trilobate process on the cephalon of the adult parasitic female. Parasites occur on the gills of the host and 2/3 of the body is buried in the cartilage of the gill arch and a connective tissue sheath secreted by the host around the parasite. In a recent study, specimens were collected from mugils in Nhlabane Estuary in Northern KwaZulu-Natal. A large number of fishes were collected and *Mugilicola* was sampled over a period of time representing all 4 seasons. Physical quality of the water was recorded. Parasites were studied and it was found that the trilobate cephalic process was not always present and when it was, the size and shape differed from pronounced to virtually absent. There was no correlation between size/age of the parasite and size or shape of the process and no correlation was found between water quality and appearance of the trilobate process. It is therefore suggested that this structure is not a constant feature. This suggestion puts a question mark over the validity of this species and even other species in this genus.

A photographic method to record the ecological changes occurring on a communal grazing area in South Africa during a 2-year field survey

N R Bryson
Department of Infectious Diseases and Public Health, Faculty of Veterinary Science, PO Box 236, Medunsa, 0204 South Africa.

The communal grazing area at Rietgat (25°23′S, 27°49′E) was visited regularly during a 2-year survey (1996–1997) to collect adult and nymphal *Amblyomma hebraeum* ticks. Previous studies indicated that there were large seasonal changes in the vegetation cover at these communal grazing areas. Difficulty was, however, experienced in recording such a complex series of events. A simple photographic method was devised to closely monitor the changes. On each visit, colour slides were taken at each of the 25 different ecological areas visited during the survey. Care was taken to visit the exact spot, and to photograph the same view on each occasion. Although there are many very complex and detailed ways of recording the seasonal changes in vegetation cover, many require expensive equipment and are time-consuming. They often only measure 1 or 2 parameters and do not give a holistic view of the complex changes that are occurring. This photographic method requires little expertise, and the results reflect complex changes. The different ecological changes, including vegetation cover, surface water, grasses, shrubs and trees, as well as the condition of the grazing animals, can all be recorded. In conclusion, a permanent record of the changes occurring on a communal grazing area can easily be made with this simple, inexpensive yet effective method.

The impact of immune evasion/suppression in the expression of cross-resistance in rabbits and calves

F C Clarke[a], D A Els[a] and J D F Boomker[b]
[a]*Department of Biology, and*
[b]*Department of Veterinary Pathology, Medical University of Southern Africa, Medunsa, Pretoria, 0204 South Africa.*

Ixodid ticks remain attached to their hosts for extended periods allowing ample opportunity for the development of immune-mediated rejection and resistance mechanisms in the host. Where parasite and host have evolved together, a mature host/parasite relationship exists and parasite numbers are limited, enabling both to coexist. The intrusion of the domestic animal results in a surrogate host/parasite relationship where the impact of the acquired immunity is partially counteracted by the feeding ticks. In the absence of a mature host/parasite relationship, as in the case of the laboratory host, the impact of the acquired immunity is pronounced. The main objective of the study was to determine the degree of cross-reactivity between the responses mounted by rabbits and cattle to the adults of some of the economically important African ticks. An immune response was, therefore, elicited in the hosts through repeated adult infestations with 1 tick species before challenging this response with an adult infestation of another tick species. Cross-resistance was obvious in all tick combinations used in the rabbit host. The protection was in some cases pronounced, and in other cases only marginal. Cross-reactivity existed in all 4 tick combinations used in the cattle hosts, but the effect could only be detected as a decrease in replete female mass.

Additional features of *Kroyeria dispar* Wilson, 1935 (Kroyeriidae, Copepoda) from the tiger shark *Galeocerdo cuvier*

S M Dippenaar and P A S Olivier
Department of Zoology and Biology, University of the North, Private Bag X1106, Sovenga, 0727 South Africa.

Copepod parasites were collected from a tiger shark caught in gill nets at Natal Sharks Board. Among the parasites collected from the gills were Eudactylinidae (Copepoda), *Echthrogaleus* (Pandaridae, Copepoda) and *Kroyeria dispar.* The parasites collected were preserved in 70 % ethanol. They were examined with the aid of a stereo microscope. Thereafter specimens were cleaned in an

ultrasonic bath, dehydrated in graded ethanol, critical point dried and sputter-coated for SEM-studies. An examination of the morphological features of *K. dispar,* with the aid of scanning electron microscopy, revealed additional features of taxonomic importance. These features include the structure of the maxillule, maxilla and maxilliped. The armature of the legs and the caudal rami were also found to be different from those described previously. Postantennal processes, an interesting feature new to Kroyeriidae, were discovered anterior to the basal processes of the maxillae. These additional, as well as previously vaguely described features, are discussed.

Prevalence of *Giardia* infection in children of five communities in South Africa

A C Evans[a], J E Fincham[a], M B Markus[b], R C A Thompson[c], V J Arendse[a] and J Mwamba[d]
[a]*National Research Programme for Nutritional Intervention, Medical Research Council, PO Box 19070, Tygerberg, 7505 South Africa.*
[b]*Zoology Department, University of the Witwatersrand, 2050 South Africa.*
[c]*Murdoch University, Murdoch 6150, Western Australia.*
[d]*Health Department, CMC, Cape Town, South Africa.*

Giardia duodenale infection is highly contagious and contractible during early infancy. Transmission is by ingestion of viable cysts. Sources can be food or drink, but most likely through intimate contact between infected and uninfected children, especially those in large families, orphanages and elementary schools. Giardiosis causes gastrointestinal tract irritation, dehydration, epigastric pain, flatulence, diarrhoea, and loss of weight and appetite. Prevention is primarily being aware of possible faecal pollution of water, and need for personal family and group hygiene. In this study prevalence of parasite infection was determined in baseline and follow-up stool surveys in young children, during nutritional assessment and monitoring programmes in 6 transitional and low socioeconomic communities. Stools were processed for microscopy using the formol-ether method. Three communities were assessed more than once. Of 2976 stools 719 were positive; mean prevalence, 18.1 % (range : 6–36 %); total children positive = 474 (range: 20–185). Some children remained infected for 2–6 consecutive 4-monthly tests over 2 years. Evidence of persistent infections prompts further investigation of typing using PCR fingerprinting methodologies.

Severe and frequent trichurosis and ascarosis in Khayelitsha children

J E Fincham[a], J C Mwamba[b], A C Evans[a] and M B Markus[c]
[a]*National Research Programme for Nutritional Intervention, Medical Research Council, PO Box 19070, Tygerberg, 7505 South Africa.*
[b]*Tygerberg City Health Service, Matthew Goniwe Clinic, Khayelitsha, 7783 South Africa.*
[c]*Parasitology Research Programme, University of the Witwatersrand, Johannesburg, 2050 South Africa.*

It is necessary to establish the prevalences and severities of infections by intestinal parasites in South African children in order to focus, plan and implement corrective prevention, and to improve nutrition. This applies especially to communities in transition where human ecology usually increases the risk of infections by parasites and other diseases. Children at a Khayelitsha primary school (n = 316) provided stool samples, permitting powerful estimates of prevalence and severity within the school. Samples were processed for microscopy by formol-ether concentration, and worm eggs were identified and expressed as eggs/gram of stool, which is an indicator of severity of infection. Prevalences were: trichurosis 84 %; ascarosis 73 % and concomitant infections 66 %. *Taenia* spp eggs were present in >2 % of samples, and cysts of *Giardia duodenalis* in 13 %. Trichurosis was often as severe as in Jamaican children in whom this infection significantly impaired growth, school attendance, mental performance and iron status. Ascarosis was usually more severe than in the West Indian children, which must increase morbidity. Prevalences of infections by both worms exceeded the limit recommended internationally as indicating a need for mass deworming. It is necessary to assess infections by intestinal parasites in disadvantaged communities, and to start corrective interventions where necessary.

The morphology of Haller's organ in the yellow dog tick (*Haemaphysalis leachi*)

E D Green[a], C Baker[b] and F C Clarke[c]
[a]*Department of Anatomy,*
[b]*Electron Microscope Unit, and*
[c]*Department of Biology, Medical University of Southern Africa, Medunsa, 0204 South Africa.*

Haemaphysalis leachi is the main vector of *Babesia canis* causing the frequently fatal canine biliary fever in dogs. As this is a 3-host tick, the micromorphology of Haller's organ, which functions in host selection, was described in each of the instars. Larvae, nymphs and adult ticks were preserved in 70 % ethanol. These were routinely processed for scanning electron microscopy and viewed in a Leica Stereoscan 420 at 5–7 kV. The morphology of Haller's organ was distinct in each instar. The larvae had only 6 sensory setae proximal to the capsule, which had a thin, slit-like opening. The anterior pit contained only 6 small sensilla of approximately the same length. In the nymph an additional pair of setae had developed proximal to the capsule, while the opening of the capsule enlarged to become oval in the adults. The number of sensilla in the anterior pit increased to 7 with the distal porous sensillum more than doubling in length. In the adults this sensory sensillum had enlarged even further and the number of external pores was greatly increased. These differences may be a result of the organ being used by the different instars for selecting different hosts, such as rodents by the immature stages and the large carnivores by the adult ticks.

SEM observations on the rare equid-sucking louse *Ratemia squamulata* (Neumann)

E D Green[a] and C Baker[b]
[a]*Department of Anatomy, and*
[b]*Electron Microscope Unit, Medical University of Southern Africa, Medunsa, 0204 South Africa.*

Ratemia squamulata was the most abundant species collected together with another common equid sucking louse *Haematopinus asini* from the same donkey. The literature showed that it is a relatively rare African species that has been previously collected on Burchell's zebra but not on horses. As the last descriptions were made 5 decades ago, a scanning electron microscopical study was performed. Specimens were combed from the lower neck and fixed in 70 % ethanol. Samples were routinely processed for scanning electron microscopy and viewed in a Leica Stereoscan 420 at 5–7 kV. The most striking feature was the 3 large, raised, trapezoid paratergal plates which included the spiracles on abdominal segments 3–6. The abdomen, previously described as membranous, was covered with overlapping scales. The robust claws of the 2nd and 3rd legs each had a distinctive notch on their anterior side. Strong, pointed, terminal spines were observed on the pretarsal sclerites which are also covered by scales for gripping the hairs of the host. The antennal sensoria consisted of a terminal peg organ with 12 sensilla, 2 pore organs and a double plate organ for orientation of this eyeless louse.

Collaborative project between University of Minnesota, Minneapolis and Amoebiasis Research Programme, South African Medical Research Council

A P Gumede, T M Shange, S G Reddy and T F H G Jackson
South African Medical Research Council, PO Box 17120, Congella, 4013 South Africa.

Invasive amoebiosis is one of the leading parasitic causes of morbidity and mortality. *Entamoeba histolytica* infects 1 % of the world's population, causing 100 million cases of invasive amoebiosis and up to 70 000 deaths annually. The aim of the current study is to

investigate and characterise the natural immune responses that develop subsequent to invasive amoebiosis and asymptomatic intestinal infection with *E. histolytica.* One hundred patients cured of amoebic liver abscess and 938 of their close associates were recruited into the study. To date, samples from all 100 index cases and their associates have been evaluated for 1 to 12 visits (every 3 months), with 70 % of subjects having completed a year of follow-up. Forty percent of subjects have completed 2 years of follow-up, and 10 % have completed the full 3 years. Study subject assessments include faecal microscopy and culture for *Entamoeba,* isoenzyme characterisation of isolates, detection of *E. histolytica* lectin antigen in faeces and serum, assay of salivary and faecal anti-lectin sIgA responses, and serum anti-lectin IgG. Demographic and clinical data are also recorded. The progress of this long-term study is discussed.

Occurrence and diversity of coccidia in indigenous, Saanen and crossbred goats in South Africa

C K Harper and B L Penzhorn
Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

Occurrence and diversity of *Eimeria* species in 2 groups of indigenous South African goats kept under traditional management systems, as well as in a mixed herd of Saanen, indigenous and crossbred goats kept under an intensive management system, were examined. Faecal specimens were collected monthly over a 13-month period from both immature (<1 year old) and adult goats. Infection rates ranged from 88.7 % to 100 % in the various groups. Mean oocysts per gram of faeces (OPG) of immature goats exceeded that of adult goats at all 3 sites. There was no consistent difference between adult OPG counts at the 3 sites. Under the intensive system, adult crossbred goats had significantly higher OPG counts than adult Saanen and indigenous goats. Overall, OPG counts of immature goats were significantly higher during the dry season (winter) than during the wet season (summer). Ten *Eimeria* species were identified, *Eimeria arloingi* being the most prevalent species at all 3 sites, followed by *E. hirci.* Up to 7 *Eimeria* species were recovered from individual specimens.

Protective immunity against *Entamoeba dispar* following *Entamoeba histolytica* infection

T F H G Jackson, A Lifson and J I Ravdin
[a]*Amoebiasis Research Programme, South African Medical Research Council, PO Box 17120, Congella, Durban, 4013 South Africa.*
[b]*Department of Medicine, University of Minnesota, Box 194, Minneapolis, Minnesota 55455, USA.*

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 amoebiosis and 100 000 deaths annually. In a prospective follow-up study of 100 index cases cured of amoebic liver abscess and 1054 of their close family associates, we are attempting to establish whether natural immunity develops after infection with these 2 parasites. All study subjects are followed for at least 3 years after enrollment in the study. Parameters such as the clinical history, faecal microscopy, serological response, zymodeme type, specific faecal antigen, specific circulating antigen and specific secretory IgA response are monitored every 3 months during this period. The specific secretory IgA response is performed on salivary samples and employs the highly conserved galactose-inhibitable lectin occurring on the surface of these parasites; this immunological response has been associated with active infection and its persistence after clinical cure seems to give rise to protective immunity. Infection with *E. histolytica* has been found to confer protection to subsequent infection with *E. dispar.* This apparent cross-immunity has important implications for the development of vaccines against these parasites.

A novel method for quantification of viable *Giardia* cysts in water samples

C Jarmey-Swan[a,b], R A Gibbs[b], G E Ho[b], I W Bailey[a] and A R Howgrave-Graham[c]
[a]*Analytical Services, Umgeni Water, PO Box 9, Pietermaritzburg, 3200 South Africa.*
[b]*Institute of Environmental Science, Murdoch University, Murdoch 6150, Western Australia.*
[c]*Department of Microbiology and Plant Pathology, University of Natal, Private Bag X01, Scottsville, 3209 South Africa.*

Assessment of *Giardia* viability is a major requirement for public health purveyors and the water industry. Several indicators of viability such as stains, excystation and animal infectivity have been used to enumerate cysts, with varying degrees of success. A combined detection/viability method for use in water samples would be useful for detecting and determining the viability of cysts in raw and drinking waters and the efficacy of disinfection at treatment plants. Distilled water samples were seeded with purified *Giardia* cysts and incubated with fluorescein diacetate (FDA) initially to stain viable cysts followed by tetramethyl red labelled anti-*Giardia* monoclonal antibodies (TMR) for confirmation of identity. As a result of FDA staining, green fluorescence of intact viable cysts was observed microscopically using a 450–490 nm exciter filter while non-viable cysts were not stained. *Giardia* cysts reacted positively with TMR and glowed red using a triple-band microscope filter with excitations of 400/495/570 nm. At these wavelengths a combination of FDA and TMR stained viable cysts green internally with a red wall while non-viable cysts only stained red. This simple, reliable and quick method allowed differentiation of *Giardia* cysts in water samples while simultaneously determining their viability.

Morphology and life history of a 27-spined echinostome parasite

P H King
Department of Biology, Medical University of Southern Africa, PO Box 139, Medunsa, 0204 South Africa.

Echinostome parasites are characterised by a collar of spines surrounding the oral sucker. An echinostome parasite with 27 spines in the collar was found in farm dams in the Free State. *Bulinus tropicus* (a freshwater snail) was found to shed cercariae bearing a collar with 27 spines. *Xenopus laevis laevis* (the African clawed toad, or platanna) found in the same waters was infected with metacercariae with a similar collar of spines. The objectives of this study were to link the 2 stages, to find the final host and to complete the life cycle experimentally. Snails were sampled with metal scoops and set out for spontaneous release of cercariae. Cercariae were used to infect laboratory-bred *Xenopus laevis,* which were subsequently used to infect a variety of experimental animals. The adult parasite was obtained from a reed cormorant, *Phalacrocorax africanus.* The parasite was identified as *Petasiger variospinosus,* the life cycle experimentally completed and all stages described by the use of light and scanning electron microscopy. This is the first report of this parasite in Africa south of the Sahara.

In vitro culture and isoenzyme analysis of *Giardia intestinalis*

Z L Kwitshana[a], S G Reddy[a], A W Sturm[b] and T F H G Jackson[a]
[a]*South African Medical Research Council, PO Box 17120, Congella, 4013 South Africa.*
[b]*Department of Medical Microbiology, University of Natal, Durban, South Africa.*

Giardia infects a large number of human and animal hosts worldwide. In humans, *Giardia* infections have variable clinical manifestations: some patients exhibit acute symptoms, a few proceed to chronic disease, while others simply remain asymptomatic. In order to establish whether parasite strain variation is responsible for this

variable pattern of pathogenesis, laboratory characterisation of this organism is necessary. The current study describes the excystment, culture and isoenzyme analysis of South African strains of *Giardia intestinalis.* Faecal specimens were obtained from giardiosis symptomatic patients at local hospitals and from asymptomatic volunteers at a children's home. *Giardia* cysts were purified on continuous 1 M sucrose gradients. Cysts were excysted *in vitro* using modified HSP-3 and acid pepsin and short-term cultures initiated. For the initiation of long-term cultures, cysts were excysted *in vivo* using a mouse inoculation model. Cysts were inoculated directly into the stomachs of neonatal C57/BL mice and trophozoites harvested from their duodenums 7 days later. Using trophozoites, 6 South African strains of *Giardia* were excysted, axenised and maintained in TYI-S-33 culture medium. Preliminary isoenzyme analysis of available isolates reveals some differences in banding patterns.

Perceptions about malaria transmission and control using mosquito-repellent plants in Mandeya: relevance to malaria control

N Lukwa[a], N Z Nyazema[b], C F Curtis[c], G L Mwaiko[d] and S K Chandiwana[a]
[a]*Blair Research, PO Box CY573, Harare, Zimbabwe.*
[b]*Medical School, A178, Harare, Zimbabwe.*
[c]*LSTMH, WC1 7HT, London, UK.*
[e]*NIMR, Box 4, Amani, Tanzania.*

Malaria is a problem in Zimbabwe where 55 % of the population resides in endemic areas. A total of 226 household heads were interviewed in May 1993 in Mandeya to assess their knowledge of malaria transmission and control. This was a cross-sectional non-intervention study where multi-stage sampling was used. A structured questionnaire was administered as an interview in a vernacular language. Over 95 % of homes were sprayed and respondents' understanding of transmission was not related to compliance with spraying. Taking control measures was related to knowledge of transmission, with 75 % of those who did not know taking no measures of their own. A total of 23.5 % people used plants because they are cheap (86 %), effective (10 %) and locally available (4 %). The leaves (86 %) of the fresh plant were crushed and burnt (72.8 %) once per day (84 %). In conclusion, the beneficiaries of the spraying programme are not well informed about its objectives nor are they fully aware of the conventional control options available.

A morphological comparison between ergasilids from two mormyrid species from the Okavango Delta

W J Luus-Powell, J P van Niekerk and P A S Olivier
Department of Zoology and Biology, University of the North, Private Bag X1106, Sovenga, 0727 South Africa.

The family Ergasilidae consists of parasitic copepods belonging to the order Poecilostomatoida. It is represented by 17 genera and over 140 species, of which more than 100 species occur on freshwater hosts. Of the genus *Ergasilus* van Nordmann, 1832, more than 100 species have been described. Only the adult females are parasitic on fish hosts. Specimens of *Ergasilus* were collected during a field trip to the Okavango Delta (Botswana). Female specimens of *Ergasilus* were removed from the gills of 2 mormyrid species, namely *Marcusenius macrolepidotus* and *Mormyrus lacerda.* Collected material was fixed in 70 % ethanol. For scanning electron microscopy, specimens were cleaned in an ultrasonic bath for 10–12 sec, dehydrated in ethanol by adding 100 % ethanol at 5-min intervals, critical point dried and sputter-coated with gold. Specimens were viewed and photographed in a JEOL 6100 scanning electron microscope at 7 kV. This study deals with the morphological detail of structures like the antennules, antennae, mandibles, maxillules, maxillae, swimming legs and caudal rami. Scanning electron microscopical differences between the ergasilids from the 2 mormyrid host species are highlighted and similarities are discussed. Scanning electron microscopy is found to be suitable when comparing morphological detail that could otherwise be overlooked when only light microscopy is applied for taxonomic purposes.

The antimalarial effect of retinoids on *Plasmodium falciparum*

M Moolman, I Havlik and L Harmse
Department of Experimental and Clinical Pharmacology, University of the Witwatersrand, 7 York Road, Parktown, Johannesburg, 2193 South Africa.

Various retinoid derivatives have recently been shown to have anti-proliferating effects on some cancer cells. It is thought that this effect is mediated via the induction of expression of the p21 and p27 cyclin-dependent kinase (CDK) inhibitory proteins. These proteins inhibit the function of cyclin-dependent kinases that are responsible for the progression of the cell cycle. Several CDK-like proteins have been identified in *P. falciparum* as well as a homologue of proliferating cell nuclear antigen which forms a complex with cyclin, CDKs and CDK-inhibitory proteins. It is not known what the effects of the various retinoids are on the erythrocytic stages of the malaria parasite. The aim of this study was to elucidate the effect of retinoic acid on the erythrocytic stage of *P. falciparum.* The chloroquine-resistant strain (FCR-3) was maintained in continuous culture and exposed to various concentrations of *all-trans*-retinoic acid and *cis*-retinoic acid over a single and a double cycle. Viability of the parasites was assessed by the ^{3}H-hypoxanthine incorporation assay. *All-trans*-retinoic acid inhibited parasite growth over a double cycle of exposure with an IC_{50} of approximately 30 mM while no inhibition was observed for the single cycle of exposure. The IC_{50} for *cis*-retinoic acid was found to be approximately 1 mM for the double cycle and 7 mM for the single cycle. The increased inhibitory effect over the double cycle indicates that the 2 agents act at the schizont or merozoite re-invasion stage of the erythrocytic cycle. These preliminary data indicate that the antimalarial properties of *cis-retinoic* acid warrant further investigation.

Control of the blackfly, *Simulium chutteri,* in the Orange River, South Africa

E Myburgh
ARC-Onderstepoort Veterinary Institute, Division of Parasitology, Private Bag X5912, Upington, 8800 South Africa.

Simulium chutteri became a pest in the Orange River after the completion of the Van der Kloof and Gariep Dams in the late 1970s. Since then substantial annual stock losses caused by the blackfly have been reported along the Orange River. The ARC-Onderstepoort Veterinary Institute (ARC-OVI) first became involved in blackfly control in 1966 and its objective has been to find an effective, environmentally safe control programme that makes use of various integrated methods to lower blackfly numbers to acceptable levels. Initial control efforts were directed at the use of water-flow manipulation, but with the expansion of irrigation this became increasingly difficult. Environmentally-safe larvicides such as *Bacillus thuringiensis* var. *israelensis* (Bti) were therefore explored. Large-scale trials were carried out with helicopter applications of Bti from Hopetown to Onseepkans. Further research conducted by the ARC-OVI resulted, in 1996, in the registration of Tamephos, which is used under high-flow conditions. These 2 larvicides are now used in the annual control programme, which is jointly conducted by the Department of Agriculture and ARC-OVI. Since outbreaks are still reported periodically, it is important that research be conducted on a continuous basis so as to make the programme even more effective.

The effect of cyclin-dependent kinase inhibitors on *Plasmodium falciparum*

K Obeng-Appiah, I Havlik and L Harmse
Department of Experimental and Clinical Pharmacology, University of the Witwatersrand, 7 York Road, Parktown, Johannesburg, 2193 South Africa.

Cyclin-dependent kinases (CDKs) have been shown to control the

orderly progression of the cell cycle in many organisms. Several CDK-like proteins have been identified in *P. falciparum* but the specific functions of each have not yet been elucidated. The aim of this study was to determine the effect of the CDK inhibitors roscovitine, olomoucine and apigenin, on the viability and cycling of the erythrocytic stage of *P. falciparum.* The chloroquine resistant strain (FCR-3) was maintained in continuous *in vitro* culture. The effect of the CDK inhibitors on parasite viability was assessed by the ^{3}H-hypoxanthine incorporation assay. Olomoucine and roscovitine showed an inhibitory effect over a double cycle with IC_{50} values of approximately 3.0 mM. No inhibitory effect was observed with single cycle assays. This indicates that the 2 CDK inhibitors exert their effect at the late schizont stage or the merozoite re-invasion stage. Apigenin, on the other hand, did not have an effect on the viability of the parasites at the concentrations tested (0.1–10 mM). In view of the results obtained, it will be worthwhile to extend the study to include more potent inhibitors of CDKs.

DNA probe classification of clinical patterns of blindness in Nigeria

A F Ogunrinade[a], S Awolola[b], D A Boakye[b] and T R Unasch[c]
[a]*Department of Medical Microbiology, University of the Witwatersrand, York Road, Parktown, 2193 South Africa.*
[b]*National Institute of Medical Research, Yaba, Nigeria.*
[c]*Department of Geographic Medicine, University of Alabama, Birmingham, Alabama, USA.*

Significant strain differences occur in the clinical patterns and the epidemiology of onchocercosis in the rainforest and savannah bioclimes of West Africa. DNA probes have been developed that hybridise specifically to forest-derived and savannah-derived parasites. We have used both the forest probe (pF-1) and the savannah probe (pSS1-BT) to map out the epidemiological patterns of *Onchocerca volvulus* at 11 different foci of onchocercosis spanning various climatic zones in Nigeria. The results indicate significant correlation with known patterns of blindness in Nigeria. We conclude that DNA probes can be used to predict clinical patterns of onchocercosis.

Morphological aspects of a *Lamproglena* sp. (Copepoda: Lernaeidae) from the African pike, *Hepsetus odoe,* from the Okavango River in Botswana

P A S Olivier, J P van Niekerk and W J Luus-Powell
Department of Zoology and Biology, University of the North, Private Bag X1106, Sovenga, 0727 South Africa.

A survey of parasites of fish from the Okavango was carried out during October 1997. The African pike, *Hepsetus odoe* (Bloch, 1794), revealed a wide spectrum of parasites, including *Apiosoma* sp. from the skin and fins, *Trichodina* sp. from the skin, fins and gills, *Tripartiella* sp. from the gills, a monogenean from the gills and a *Lamproglena* sp. from the gills. To date the only published parasite records from the pike are *Myxobolus africanus* from the gills, opercular muscle and gall bladder, *Trichodina magna* from the skin, fins and gills, *Hemitrichodina robusta* from the skin and fins and *Ergasilus mirabilis* from the gills. Hosts were sampled using gill nets and transferred to temporary holding facilities to keep them alive as long as possible. Gills were removed and examined for parasites, which were fixed in 70 % ethanol. For scanning electron microscopy, specimens were cleaned in an ultrasonic bath, dehydrated in graded ethanol, critical point dried with CO_2 in absolute ethanol, sputter-coated, viewed and micrographed with a JEOL 6100 at 5–7 kV. This report deals with the morphology of a *Lamproglena* sp., a 1st record from *H. odoe* and possibly a new species. Morphological features are discussed. Attention is given to the morphology of taxonomically important features of the appendages. This species distinctively displays somatic segmentation as in *Pseudolamproglena* Boxshall, 1976. The cephalic shield forms prominent lateral lobes extending ventrally and a number of scattered sensilla are present on the surface of the cephalic shield. Morphological features of the male are also discussed with special reference to the morphology and armature of the legs.

Cowdria ruminantium infection in an African nature reserve

T F Peter[a], N R Bryson[b], B D Perry[c], C J O Callaghan[d], G F Medley[d], G M Mlambo[a], G E Smith[a], I G Horak[e], M J Burridge[f] and S M Mahan[a]
[a]*UF/USAID/SADC Heartwater Project, Veterinary Research Laboratories, Harare, Zimbabwe.*
[b]*Department of Infectious Diseases and Public Health, Faculty of Veterinary Science, PO Box 236, Medunsa, 0204 South Africa.*
[c]*International Livestock Research Institute, Nairobi, Kenya.*
[d]*Department of Biological Sciences, University of Warwick, Coventry, United Kingdom.*
[e]*Department of Veterinary Tropical Diseases, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.*
[f]*Department of Pathobiology, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA.*

Amblyomma hebraeum is the main vector of heartwater, an economically important disease of ruminants caused by *Cowdria ruminantium* in southern Africa. Recent work has indicated that wild animals play an important role in the epidemiology of the disease. There are, however, few reports from game reserves on the isolation of *C. ruminantium,* the rickettsial agent causing heartwater, from the reservoir hosts or the ticks. The objectives of this study were to collect adult *A. hebraeum* ticks from the Kruger National Park, isolate *C. ruminantium,* and determine the prevalence of *C. ruminantium* in the ticks. Adult *A. hebraeum* ticks (n = 385) were collected using pheromone traps in Kruger National Park. A sample (n = 60) were fed on a susceptible goat. These ticks transmitted *C. ruminantium* to the goat, resulting in acute, fatal heartwater. *C. ruminantium* was isolated in bovine endothelial cell culture from the plasma of this animal during the febrile stage of the disease and was subsequently transmitted to a susceptible goat, causing heartwater. The prevalence of *C. ruminantium* infection in the ticks was determined by PCR analysis to be 1.7 % (95 %, CI 0.71–4.0 %, n = 292). DNA probe analysis, which is less sensitive than PCR, detected infection in 3 of the 5 PCR-positive ticks. The remaining infections were below the detection limit of the DNA probe, which is approximately 70 000 organisms. This is the first evidence of a vector/wildlife cycle of *C. ruminantium.*

To determine the effect of rifampicin and ethambutol on the malaria parasite *Plasmodium falciparum*

M L Phillips, R L van Zyl, I Havlik and L Harmse
Department of Experimental and Clinical Pharmacology, University of the Witwatersrand, 7 York Road, Parktown, 2193 South Africa.

Owing to the increase of resistance to antimalarial drugs there is a need for alternative agents for the treatment of malaria. Many people in South Africa are at risk of contracting tuberculosis and malaria. The antimalarial activity of rifampicin, an inhibitor of bacterial RNA polymerase, has not been fully investigated. There are no reports in the literature on the effect of ethambutol on malaria. The mechanism of action of ethambutol is still uncertain but it appears to interfere with the synthesis of RNA and the mycobacterial cell wall. The aims of this study were, therefore, to determine whether rifampicin and ethambutol have an inhibitory effect on the chloroquine-sensitive and resistant strains of *P. falciparum.* Drug sensitivity assays were performed on the chloroquine-resistant strain, (FCR-3) using the ^{3}H-hypoxanthine incorporation assay to calculate the respective IC_{50} values. An IC_{50} of 0.46 mM was obtained for the single cycle of rifampicin while ethambutol was not effective. Preliminary experiments indicate that ethambutol is effective over the double cycle with an IC_{50} of approximately 4.5 mM. The antimalarial effect of rifampicin and ethambutol may have important clinical implications where malaria and tuberculosis infections are prevalent.

Antimalarial activity in crude extracts of South African plants

M J Pholo, I Havlik, L Katsoulis and R L van Zyl
Department of Experimental and Clinical Pharmacology, University of the Witwatersrand, 7 York Road, Parktown, Johannesburg, 2193 South Africa.

In the last century quinine derived from *Cinchona* bark and artemisinin derived from *Artemisia annua* leaves have been used in the treatment of malaria. The effectiveness of these traditionally-used extracts prompted further investigation into plants used by traditional healers for malaria treatment. The aim of this study was to screen plants used by South African traditional healers for inhibitory activity against chloroquine-resistant *Plasmodium falciparum.* Interviews with traditional healers from Johannesburg regarding plants used against malaria were conducted and 9 different species were selected for screening. The plant materials were ground into fine powder, crude extracts were then prepared and lyophilised. The osmolarity of all the extracts was adjusted to 300 mOsm before testing. Chloroquine-resistant *P. falciparum* (FCR-3) malaria parasites were continuously cultured *in vitro.* Tritiated hypoxanthine incorporation assays were performed on the crude extracts to evaluate their antimalarial properties. Preliminary data of 4 plants selected indicate promising anti-malarial activity.

The effects of curdlan sulphate on the *in vitro* development of *Plasmodium falciparum*

C D Ross, I Havlik, Y Kaneko, L Harmse and R L van Zyl
Department of Experimental and Clinical Pharmacology, University of Witwatersrand, 7 York Road, Parktown, Johannesburg, 2193 South Africa.

The rise in malaria incidence and drug resistance have driven the search for novel antimalarial compounds. Curdlan sulphate (CRDS), a sulphated polysaccharide, possesses promising antimalarial properties. The aim of this study was to investigate the *in vitro* effects of CRDS on the different stages of the life cycle of the malaria parasite. The chloroquine-resistant (FCR-3) *P. falciparum* strain was maintained continuously in culture and adjusted to a 5 % haematocrit and 2 % parasitaemia for experiments. Parasites in the schizont stage were exposed to various CRDS concentrations for 6, 18 and 24 hours, with the percentage parasitaemia and stage distribution being quantified from Giemsa-stained thin blood smears for approximately 140 hours. Viability was determined from the ability of the parasite to incorporate ^{3}H-hypoxanthine. CRDS has no antimalarial activity over a single growth cycle, but has an inhibitory effect with an IC_{50} of approximately 5.1 mg/mℓ over a double cycle. Preliminary data indicated that, following 18 or 24 hours of CRDS treatment, the progression of parasite growth was halted in the schizont stage. Minimal inhibition was observed after 6 hours of CRDS treatment at 10 and 50 mg/mℓ, but inhibition at the schizont stage was increased at 100 mg/mℓ. Stage progression following CRDS removal after 6 hours resembled that of the untreated controls. The ability of the parasite to resume its normal cycle was reduced following the removal of CRDS after 24 hours exposure to high CRDS concentrations (250 and 500 mg/mℓ). CRDS, with its low toxicity profile, warrants further research, especially as part of adjunct therapy with classical antimalarial agents.

Construction of parasite distribution maps and their use for control measures

R Schuster
Institute of Parasitology and Tropical Veterinary Medicine, Free University Berlin, Koenigsweg 67, 14163 Berlin, Germany.

The host is the first-level environment or habitat for the parasite and the biotope where the host lives can be considered the second-level environment for the parasite. While some parasites have a worldwide distribution, others are restricted to particular areas depending on the occurrence of specific host categories. Knowledge of the distribution of a specific parasite of economic and/or medical importance is one of the main conditions for a successful control programme. First parasite distribution maps in Germany were drawn for fasciolosis and hypodermosis. State-controlled control programmes led to the elimination of hypodermosis in cattle in the former German Democratic Republic within 4 years. The prevalence of cattle fasciolosis decreased from an average of 30 % to lower than 1 %. By identifying the origin of infected cattle and treating cattle lungs after slaughter, an outbreak of hydatidosis in cattle in central Germany in the 1980s was stopped. Dicrocoeliosis was introduced to the Brandenburg State of Germany via infected sheep in the 1960s, and in suitable biotopes with a high land snail density and susceptible ant populations, the prevalence of dicrocoeliosis in sheep rose to 95–100 % with an average burden of 2000 flukes per animal. An abattoir survey combined with snail collections was helpful to identify places where the infection had taken place. Multilocular echinococcosis and opisthorchiidosis are 2 new zoonoses that recently occurred in the northern part of Germany. Using red foxes, the main source and the main natural reservoir, respectively, as indicators for the occurrence of both diseases, maps were drawn to forecast possible human infections. A successful control programme was carried out in the case of fox echinococcosis by using praziquantel baits.

Comparative assessment of Robinson's and TYSGM-9 culture media for the isolation and short-term growth of *Entamoeba histolytica* and *Entamoeba dispar*

A F Sumad, S G Reddy, C B Anderson, S Suparsad and T F H G Jackson
South African Medical Research Council, P O Box 17120, Congella, 4013 South Africa.

The diagnosis of amoebiosis by microscopic identification is insensitive and unable to distinguish the invasive parasite *E. histolytica* from the commensal parasite *E. dispar.* Although newer techniques like PCR and antigen detection ELISAs for the identification of *E. histolytica* are available, primary culture and isoenzyme analysis of these parasites are still used by many as an aid to diagnosis of invasive disease. Primary cultures in both Robinson's and TYSGM-9 media are xenic and contain starch as a carbohydrate source. The former is biphasic and includes a saline agar slope, while the latter is monophasic medium containing gastric mucin. This study sets out to determine which medium gave the highest yield of positive cultures from faecal samples. More than 3000 faecal samples were inoculated into both media and the cultures monitored for growth by microscopic observation of temporary wet-mount preparations. Positive cultures were harvested and resulting lysates subjected to isoenzyme electrophoresis. Using the resulting zymodemes, the organisms were classified as either *E. histolytica* or *E. dispar.* The efficacy of these methods to support short-term culture of *E. histolytica* and *E. dispar* are discussed.

A simple and effective method for the cryopreservation of axenic *Entamoeba histolytica*

S Suparsad, S G Reddy and T F H G Jackson
South African Medical Research Council, PO Box 17120, Congella, 4013 South Africa.

Successful methods for the cryopreservation of axenic *E. histolytica* have been reported in the past. However, not all investigators enjoy equal success using these methodologies. Furthermore, some methods require sophisticated equipment not readily available to all investigators. The current work outlines a simple technique for the cryopreservation of axenic *E. histolytica.* A 72-hour culture of axenic *E. histolytica* in TYI-S-33 was briefly chilled at 4 °C and gently centrifuged. The resulting trophozoite pellet was resuspended in 1 mℓ of fresh TYI-S-33 medium and transferred to a cryovial. An equal volume of an 18 % dimethyl sulphoxide solution in TYI-S-33 was added with gentle mixing. The cryovials were immersed in isopropanol in a Nalgene Freezing Chamber and the entire chamber

stored at –70 °C for 5 days. Thereafter, vials were removed and stored in liquid nitrogen indefinitely. Trophozoites were retrieved by quick-thawing at 37 °C for 2 min and transferring to pre-warmed TYI-S-33 medium. Following 30 min of incubation, the viability and motility of the trophozoites were assessed and found to be consistently greater than 80 %. Reproducible results were obtained with 3 axenic strains of *E. histolytica.*

Scanning electron microscope study on the morphology of an *Ergasilus* from *Synodontis nigromaculatus* from the Okavango Delta

J P van Niekerk, W J Luus-Powell and P A S Olivier
Department of Zoology and Biology, University of the North, Private Bag X1106, Sovenga, 0727 South Africa.

The parasite copepod *Ergasilus* van Nordmann, 1832 (Ergasilidae: Poecilostomatoida) is represented by more than 100 described species of which the majority occur on freshwater fishes. A female *Ergasilus* possesses strong prehensile antennae that it uses to attach itself around the gill filament of its fish host. Specimens of *Ergasilus* were collected in the Okavango Delta (Botswana) from *Synodontis nigromaculatus.* Collected material was fixed in 70 % ethanol. For scanning electron microscopy, specimens were cleaned in an ultrasonic bath and dehydrated by adding 100 % ethanol at 5-min intervals. Selected specimens were critical point dried with CO_2, mounted on steel pins and sputter-coated with gold. Specimens were viewed and photographed in a JEOL 6100 scanning electron microscope at 7 kV. The study deals with the ultrastructural morphology of the antennule, antenna, mandible, maxillule, maxilla, setation on swimming legs, caudal rami and body ornamentation. The importance of these structures for taxonomic purposes are discussed.

The effect of ferric and ferrous ion-chelating agents on the *in vitro* growth of *Plasmodium falciparum*

R L van Zyl and I Havlik
Department of Experimental and Clinical Pharmacology, University of the Witwatersrand, 7 York Road, Parktown, 2193 South Africa.

The human host implements a status of 'nutritional immunity' by chelating free iron to inhibit essential iron-dependent biochemical reactions in the parasite and thereby inhibit parasite growth. Iron-chelating agents are used to mimic this effect and to inhibit the growth of *P. falciparum.* Combination studies, involving chelating agents, have concentrated on the ferric ion-chelating agents. Thus, the aim of this study was to investigate the combined effect of ferric and ferrous ion-chelating agents on the *in vitro* growth of *P. falciparum* malaria. The *in vitro* activity of various ion-chelating agents was evaluated against a chloroquine-resistant *P. falciparum* (FCR-3) strain using the ^{3}H-hypoxanthine incorporation drug sensitivity assay. The chelating agents inhibited parasite growth with IC_{50} values ranging from 15.11 ± 3.00 μM to 25.33 ± 8.14 μM. The combination of 2 ferric ion-chelating agents, desferrioxamine and desferrithiocin, resulted in an additive interaction, while a synergistic interaction was noted between 2 ferrous ion-chelating agents, 2.2-bipyridyl and bathophenanthroline sulphonate. The combination of ferrous and ferric ion-chelating agents resulted in variable interactions, depending on the lipophilicity of the ferrous ion-chelating agent. The antimalarial activity of an iron-chelating agent is greatly influenced by its iron-binding constant and lipophilicity. The combination of iron-chelating agents might be of therapeutic value in the treatment of malaria and warrants further investigation.

Evaluation of biological assessment indices in the Olifants River system

R M Watson and A Avenant-Oldewage
Department of Zoology, Rand Afrikaans University, PO Box 524, Auckland Park, Gauteng, 2006 South Africa.

Limited success with point-source control as a measure of the health of an aquatic ecosystem is primarily the reason for an international shift towards the use of biological monitoring.

The present study evaluates 4 sample sites, 2 dams and 2 river points, in the Olifants River system. The health assessment index (HAI) and a newly-developed parasite index were used as the biological monitoring systems, and can be seen as quantitative indices that enable statistical comparison of various fish species, namely *Clarias gariepinus, Oreochromis mossambicus* and *Barbus marequensis,* in 4 different water bodies.

Results from the HAI clearly showed that Loskop Dam was more polluted than Bronkhorstspruit Dam, while Mamba was more polluted than Balule. The values attained for blood and liver tissue, as well as the types of parasites present, were the most indicative parameters in the HAI.

Parasites (one of the HAI variables) were used independently of the HAI, to determine whether they alone can be indicators of pollution levels, and it was found that parasites are good indicators of environmental health. Ectoparasites and endoparasites were evaluated separately and it was found that at highly polluted sites, ectoparasite numbers decreased and endoparasite numbers increased.

Analysis of heavy-metal concentrations using the atomic absorption spectrometer substantiated the results obtained using the HAI and parasite index. There was a good correlation between the findings of the HAI, parasite loads and the heavy-metal values at all 4 sampling sites.