Parasitological Society of Southern Africa

The following are the abstracts of papers presented at the Annual Meeting of the Society, held at the Rand Afrikaans University, Johannesburg, on 28 and 29 June, 1984.

Parasitologiese Vereniging van Suidelike Afrika

Die uittreksels van referate wat tydens die jaarlikse vergadering van die Vereniging, op 28 en 29 Junie 1984 by die Randse Afrikaanse Universiteit, Johannesburg, aangebied is, word hieronder aangegee.

Nematodes of Kudu, *Tragelaphus strepsiceros*, from the Kruger National Park

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During a survey of the parasites of wild animals in the Kruger National Park, 96 kudu, *Tragelaphus strepsiceros*, were culled in the southern part of the park and their helminths collected. Two trematode species, four cestodes and 18 nematodes were recovered, of which *Haemonchus vegliai, Trichostrongylus* spp. and *Cooperia* spp. were recovered regularly throughout the survey period of 24 months.

The largest number of helminths recovered from a single kudu was 7279, and the largest mean monthly burden was 5163, both recorded in March 1982. The smallest number was 54, recovered from a calf 6 months old in June 1982, and the smallest mean monthly burden was 797 recovered in November 1981.

The mean number of helminths increased in male animals from 1460 in animals less than one year old to 2833 in mature kudu, four or more years old. Helminth counts were fairly high in young female calves (1855), lower in females 1 – 2 years old (512), and this increased gradually to 2794 in females of four years and older.

The worm burdens in adult female kudu also varied. Pregnant females had a mean burden of 1943 worms, reproductively inactive females had 2505 and lactating females had 5796. The last count is probably the result of the stress of caring for the calf and lactation.

Because the sample sizes were small, the above figures could not be statistically analysed.

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Vultures *Gyps* Spp. as Final Hosts of *Sacrocystis* of the Impala *Aepyceros melampus*

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Sporocysts of *Sarcocystis* were found in the faeces of wild, naturally infected vultures for the first time by Markus and Mundy [(1979). *Parasitology* 79, xxxix]. The authors concluded that 'vultures are probably final hosts of more than one species of *Sarcocystis* of large game animals'.

Subsequently, four captive vultures which did not have intestinal sarcocystosis were used in a transmission experiment involving *Sarcocystis*-infected skeletal muscle from the impala *Aepyceros melampus*, obtained in August 1980 in the Sengwa Wildlife Research Area of north-west Zimbabwe (18°08'S, 28°12'E). One Cape Vulture *Gyps coprotheres* and one White-backed Vulture *G. africanus* each received five 0.5 kg meals of this venison at two-day intervals. Another Cape Vulture and a second White-backed Vulture served as controls and were fed their normal diet of bovine heads and rabbits which had been deep-frozen. After a prepatent period of 15 – 16 days (from the first infective meal), both experimental birds started shedding *Sarcocystis* sporocysts in their faeces. The faeces of the control vultures, on the other hand, remained *Sarcocystis*-negative throught the experiment.

Two species of *Sarcocystis* were detected by light and electron microscopy in samples of the impala meat ingested by the experimental vultures. One parasite had cyst wall protrusions flattened along the surface of the cyst, while the other species of *Sarcocystis* could be distinguished by its characteristic mushroom-shaped cyst wall protrusions. Whether both or only one of these organisms infected the vultures is not known.

It has hitherto been thought that carnivorous mammals are the final hosts of all *Sarcosystis* species of large ungulates. However, from the results of our experiment it has become evident that at least one species of *Sarcocystis* of a large herbivorous mammal has an avian final host.

This work was supported by the Endangered Wildlife Trust and the CSIR. The vultures used in the experiment were part of a group cared for at Larvon Bird Gardens, Harare, Zimbabwe.

Morphological Studies of Nematodes in Zebras

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Equids are hosts for 100 known species of helminths, primarily nematodes. The nematodes are represented by 8 families and one of these, the Atractidae, may number millions in an individual zebra. Another family, the Strongylidae, is present in burdens of tens of thousands or more in all equids. In this, the first detailed study of the nematodes of zebras, four new species of strongyle and two new species of spirurid were found. These families of nematodes have diverse morphological features and much variation also exists between species.

While two new nematode species were easily recognized and described, several genera have posed other identification problems. Early descriptions based on just one or two specimens as well as brevity of the description have made identification of the genus *Cylindropharynx* challenging. Eight known species were differentiated by the morphology of the male genital appendage. Closer examination reveals there is a great variation of this appendage and this has not been recorded previously. The identification of *Triodontophorus* based on mouth denticulation reveals a wide variation in the serration of teeth.

Some Observations Made on the Parasitic Infections of Black Pupils in the Eastern Lowveld

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Preliminary studies were made of parasite ova in the urine and stools of primary school pupils aged 6 – 18 years old from eight schools in three different areas of the eastern lowveld: black resettlement (BR), 4 schools; black urban (BU), 1; white farming (WF), 3. Differences in the prevalence of *Schistosoma haematobium, S. mansoni, S. mattheei, Ascaris lumbricoides*, hookworm, *Taenia* spp., *Strongyloides stercoralis, Trichuris trichiura* and *Hymenolepis nana* were compared within the age groups '10 years and under' and 'over 10 years'. The present length of abode and area or place or origin of each child were recorded. The 'over 10 years' age group contributed more to *S. haematobium, S. mansoni*, hookworm, *T. trichiura* and *Taenia* spp. and less to *A. lumbricoides* and *H. nana* infection rates.

Leucocytozoa of Gallinaceous Birds in Southern Africa

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The paper deals with Leucocytozoa of two introduced species, the domestic fowl *Gallus gallus* and the turkey *Meleagris gallopavo*, and of two indigenous species, the helmeted guineafowl *Numida meleagris* and Swain-

son's francolin *Pternistis swainsoni*. Of the former the turkey appears to have brought its own spindle-shaped *Leucocytozoon, L. smithi*, whereas the spherical *L. schoutedeni* of the fowl appears to be limited to Africa. Spindle-shaped *L. neavei* is generally believed to be shared by guineafowl and francolin. The latter, however, also carries a form which is very similar to *L. smithi* of the turkey as well as another spindle-shaped giant form which has been named *L. peaolopesi*, and a spherical form similar to *L. schoutedeni*. In the guineafowl a free spherical form is occasionally seen. Four questions arise from these findings: Which is the original host of *L. schoutedeni*? If all the forms found in the francolin are of one species, can this still be *L. neavei*, or do the different forms represent different species? In this case, does *L. smithi* infect francolins? Is the free spherical form seen in the guineafowl *Akiba caulleryi*?

Implications of Chemotherapy of *Theileria lawrencei* Infections (Corridor Disease) in Cattle

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It is firmly believed that *Theileria lawrencei* infection of cattle, (buffalo disease, or commonly known as Corridor disease), is associated with the presence of buffalo (*Syncerus caffer*) in South Africa. Sporadic outbreaks of this fatal disease among cattle occur on farms bordering the Kruger, Hluhluwe and Umfolozi game reserves. A few isolated outbreaks have also been reported from farms in the northern and north-eastern Transvaal where private nature reserves accommodate buffalo. Buffalo remain carriers of this parasite for long periods and infection of *Rhipicephalus appendiculatus* feeding on buffalo apparently occurs readily.

Laboratory observations in South Africa have shown that splenectomized cattle can also become carriers. More recently it was demonstrated that an intact bovine which recovered after treatment with halofuginone developed a carrier status. Ticks fed on this animal transmitted the infection transstadially, indicating that direct transmission between cattle can take place. The application of effective chemotherapy during Corridor disease outbreaks may result in establishing carrier status in recovered cattle and this may eventually have far-reaching effects on the epidemiology of the disease in South Africa.

Distribution and Prevalence of Avian Schistosomes in South Africa

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Although the eggs of seven species of schistosome (Trematoda) have been recovered from South African birds, two stand out as being both widespread and common in this country. These are a species of *Trichobilharzia*, parasitic in waterfowl, particularly the Spurwing Goose, and a species of *Gigantobilharzia*, which infects coastal birds, especially the Kelp Gull. *Trichobilharzia* sp., whose intermediate host is the snail *Lymnaea natalensis*, infects 60% of Spurwing Geese in the Transvaal and Natal but is absent from the south-western Cape. *Gigantobilharzia* sp. infects coastal birds around much of the coastline but the highest prevalence rates occur in the south-western Cape. The intermediate host of this parasite is not known.

Dermatitis caused by the penetration of human skin by the cercariae of these flukes is therefore likely to be contracted in both fresh and marine waters. Clinically this is only a transitory phenomenon but false positive diagnoses of schistosomiasis could occur when immunological tests are used.

Human Water Contact Patterns in Relation to Schistosomiasis Transmission

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A study was made of human water contact patterns in a river running through an informal settlement near an industrial area. The resultant exposure indices were correlated with the prevalence of schistosomiasis over age-sex classes in the area. Because it is now agreed that a control programme for schistosomiasis in an endemic area should be preceded by a study of the human water contact patterns, the primary aim of the study was to provide guidelines for control of the disease. The second aim was methodological: to develop cost-effective methods which could be used reliably in other areas where water contact patterns are different.

Results from observations and interviews indicated that a relatively small number of children who went swimming played a disproportionately large part in the transmission cycle. The washing of clothes and blankets was the second major water contact activity. An exposure index which included a measure of body surface area was compared with an index which was based simply on frequency and length of contact. The former was found to be a better predictor of prevalence of schistosomiasis in the area. This index had a high correlation with the prevalence of *S. haematobium* over age-sex classes (R_s = 0.900), whereas the prevalence of *S. mansoni* was less well-correlated (R_s = 0.498).

Animal Models for Schistosomiasis *mansoni*: A Comparative Study of Two Rodent Species

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Over the years in this country, *Mastomys coucha* has proved to be an extremely valuable rodent host for maintaining a wide range of mammalian schistosomes. However, its usefulness as a representative animal model for the study of human hepatic schistosomiasis has not been adequately investigated. The present study was embarked on to compare the response of *M. coucha* to *Schistosoma mansoni* infected with that of the animal model most commonly employed in schistosomiasis research, namely the laboratory mouse.

M. coucha and BALB/c mice were exposed to cercarial numbers such that worm: body weight ratios were similar. Several features characterised the differences in the response of these two rodent species to *S. mansoni* infection. These included mortality rates and splenic responses as well as liver and gastrointestinal pathology. BALB/c mice presented a pathological picture much more analogous to that found in man in that they developed characteristic fibrosis in the portal areas with the formation of fibrous septae accompanied by marked splenomegaly. To date, none of these features have been noted in *M. coucha*. The results of this study have highlighted the importance of accurately assessing host/parasite relationships prior to the selection of an animal model.

Parasitological Consequence of Introducing Foreign Fish Species

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During the last century, various fish species were introduced into South Africa with the aim of establishing inland aquaculture. The introduced fish species have been responsible for the distribution of foreign fish parasites throughout Southern Africa.

The fish tapeworm *Bothriocephalus*, originating from the East, has been distributed worldwide along with the grass carp. This tapeworm was responsible for large-scale mortalities at the Lowveld Fishery Station and has since been found in various localities in the Transvaal. The fish louse *Argulus japonicus*, which has a worldwide association with the common carp, was found in dams in the western Transvaal, where all fish examined were found to be infested by as many as 500 parasites per fish. Although effective treatment of these parasites can be obtained under fishery conditions, the treatment of parasites in large dams is impossible. The long-term effect of introduced parasites on the fish populations in Transvaal is discussed and the desirability of introducing exotic fish species is questioned.

Feeding Mechanism of the Fish Ectoparasite *Dolops ranarum* (Crustacea: Branchiura)

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Three genera representative of parasitic Branchiura occur on freshwater fish in Southern Africa. *Dolops ranarum* are found mainly on *Oreochromis mossambicus* and *Clarias gariepinus* and are widely distributed in the Transvaal. Although skin lesions are caused by this parasite, the mechanism of feeding and damage to its host is not yet fully understood. In order to elucidate these points, the morphology of the

mouthparts and feeding structures were studied and compared with those of *Argulus.*

Morphology and Ultrastructure of Fish Ectoparasites of the Genus *Trichodina* (Ciliophora: Trichodinidae)

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Under winter conditions, representatives of the genus *Trichodina* have, in various parts of the world, been responsible for large-scale fish mortalities. Although the taxonomy of these parasites has been studied extensively, very little is known about the morphology, mechanism of feeding and attachment to the host. The morphology of the attachment structure was studied by means of light and electron microscopy and the mechanism of feeding and damage to the host described.

Occurrence of the Fish Louse, *Argulus japonicus*, in the Western Transvaal

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During surveys for fish parasites in the western Transvaal, it was found that all specimens of the various fish species collected from Bloemhof Dam and Lake Barberspan were infested by *Argulus japonicus* Thiele, 1900. Infestation statistics revealed that *A. japonicus* is not specific to any host or attachment site on hosts. *A. japonicus* has a worldwide distribution associated with exotic cyprinids such as the carp and goldfish. This parasite is not indigenous to Africa and was probably introduced into Southern Africa by the introduction of the carp or other cyprinids.

A Case of Aquatic Hyperparasitism

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In the course of studies on fish parasites, crustacean ectoparasites such as *Lernaea barnimiana, L. cyprinacea* and *Dolops ranarum* were found to parasitise freshwater fish in South Africa. These crustacean ectoparasites were in turn found to be infested by sessile Ciliophora of which seven species, including three new species, are described. Of the sessile Ciliophora species infesting fish, some were found to be infested by endoparasites of the genus *Endosphaera.* These protozoan parasites were found to be host-specific, so that species found on the crustaceans did not infest the fish.

Epizootiology of the Helminth Fauna of the Digestive Tract of the Chacma Baboon, *Papio ursinus* (Kerr, 1792), from Different Localities in the Transvaal

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One hundred and eleven baboons from the Loskop Dam, Suikerbosrand and Scrutton nature reserves and the Sabie-Tweefontein forest reserve were examined for helminths of the digestive tract. The helminths recovered were *Bertiella studeri, Enterobius vermicularis, Oesophagostomum bifurcum, Physaloptera caucasica, Streptopharagus pigmentatus, Strongyloides fülleborni, Trichostrongylus falculatus, Trichuris trichiura* and females of *Trichuris,* which possibly belong to a new species. Most baboons harboured three, and some as many as six, species of helminths. Worm burdens of the various helminths varied greatly even among baboons from the same locality, age group and sex. All the helminths found in the present study can occur in very young animals. Worm burdens generally increased as the host aged, with a subsequent decrease among adult baboons of *E. vermicularis, S. fülleborni* and *T. falculatus.* Higher worm burdens in the wet season were found for *B. studeri* and *O. bifurcum,* whereas *T. falculatus* was found in higher numbers during the dry season. No significant differences in worm burdens were found in male and female baboons, but *P. caucasica* was more prevalent in males. *T. falculatus* and *E. vermicularis* are new records for the Chacma baboon.

Dipetalonema Species in Zimbabwe

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The diagnosis of *Dipetalonema perstans* infection in man depends on the demonstration of unsheathed, non-periodic microfilariae in the blood stream. This paper describes an unsheathed, apparently periodic microfilaria from the blood of a 50-year-old woman from Mutare on the Mozambique border. The morphology of the microfilaria differs from that of *D. perstans* and *D. semiclarum* in several features, the most notable being its short length and the presence of two large clear bands, one in the anterior and one in the posterior regions of the body. Studies are in progress to investigate the pathogenicity of this parasite, and the prevalence of microfilariae in patients from Mutare.

Piscivorous Birds as Carriers of Helminth Parasites of South African Indigenous Freshwater Fish Species

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During the last decade a considerable amount of research has been undertaken on the helminth parasites of indigenous South African freshwater fish. In contrast to this little or no work was done on the role played by birds as carriers of fish-related helminth parasites, acting either as final hosts or as second intermediate hosts for these parasites. The present paper focuses on the families Phalacrocoracidae and Anhingidae as possible carriers of fish helminths.

Two species of the genus *Phalacrocorax* (*P. carbo* and *P. africanus*) and one species of the genus *Anhinga* (*A. rufa*) are discussed. Helminths isolated from these birds in the Transvaal include, amongst others, representatives of the genera *Euclinostomum, Clinostomum, Diplostomum, Nephrostomum* (Trematoda: Digenea); *Contracaecum* (Nematoda: Ascaroidea) and *Ligula* (Cestoda: Diphyllobothriidae).

Clinostomiasis in the Northern Transvaal

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Clinostomiasis is a condition caused by the infection of fish with metacercariae of trematodes of the family Clinostomatidae (Trematoda: Digenea). This condition seems to be widespread in wild fish stocks in the northern Transvaal (Limpopo and Olifants drainage systems). Twenty-eight species of freshwater fish from various localities and habitats have already been examined for trematode infections. Of these, 13 species harboured clinostome parasites. Four clinostomes from three different host species, viz. *Clinostomum tilapiae, C. van der horsti, Euclinostomum dollfusi* and *E. heterostomum,* were identified to species level. Metacercariae from the other host species were then identified to genus level only. Final taxonomic allocation will be made only after the adult morphology has been established through experimental infections in avian hosts. The extent of infection, geographical distribution as well as the effect of infections on the condition factor of the various fish hosts are discussed.

The Bio-cycle of *Ichthyophthirius multifiliis* and its Range in the Transvaal

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One of the most important parasitic diseases of freshwater fishes is 'white spot', caused by *Ichthyophthirius multifiliis.* A description is given of the bio-cycle as it is commonly found and it is contrasted with a modified cycle as proposed by Butcher [Butcher (1943). *Aust. Zool.* **10**, 125 – 137]. The significance of this is discussed, especially as regards how this influences the treatment programme of infested fish.

Investigations have been undertaken to determine the prevalence of *Ichthyopthirius multifiliis* in the Transvaal. Only two cases of naturally infested fish were found in the dams and rivers of the Transvaal — at Luphephe-Nwanedzi Twin Impoundments in Venda and in Hartbeespoort Dam. Parasites were also found at pet shops — possibly a result of importation of exotic species for aquarists. The fish breeding stations in the Transvaal was also visited; *Ichthyophthirius* was found at Marble Hall and also at the fish breeding station at Lydenburg. Reasons for the

low prevalence in natural waters, but high prevalence at the breeding stations, are discussed.

Chemotherapy of Experimental Besnoitiosis in Rabbits

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Twenty-four rabbits were experimentally infected with a bovine field isolate of *Besnoitia besnoiti*. The rabbits were infected intraperitoneally with 10^6 organisms from a VERO-cell culture. All animals developed a pronounced febrile reaction from day 5 after infection. Twelve animals were treated on the day of infection with 30 mg/kg long-acting oxytetracycline i.p. All the untreated controls developed a pronounced scrotal oedema and orchitis, indicating protection by the drug against clinical manifestations of besnoitiosis in rabbits.

Monogenetic Parasites of Freshwater Fish in South Africa

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Eleven *Barbus* species of different age-groups and sexes were seasonally collected and examined for monogenetic parasites over a period of thirty months from several dams and rivers in Lebowa and Venda. Eight *Dactylogyrus* species and a *Neodiplozoon* were recovered from their gills. Three of the *Dactylogyrus* spp., *Dactylogyrus teresae, D. enidae* and *D. dominici,* are new. New hosts and distributions are recorded for *D. afrosclerovaginus* Paperna, 1973; *D. allolongionchus* Paperna, 1973; *D. afrolongicornis afrolongicornis* Paperna, 1973; *D. afrolongicornis alberti* Paperna, 1973; *D. spinicirrus* (Paperna & Thurston, 1968) and *Neodiplozoon polycotyleus* Paperna, 1973. These parasites appear to be host specific since each of them is found only on a particular host species; thus *D. afrosclerovaginus* is found only on *Barbus paludinosus, D. allolongionchus* on *B. trimaculatus, D. afrolongicornis afrolongicornis* on *B. trimaculatus, D. afrolongicornis alberti* on *B. trimaculatus,* and *D. spinicirrus* on *B. marequensis.* However, on reviewing the monogenetic parasites of freshwater fish in Africa, it becomes apparent that these parasites are widespread in Africa but select different host species within the family Cyprinidae, in different areas. *Dactylogyrus myersi* Price, McClellan, Druckenmiller & Jacobs, 1969, is the only species which seems to parasitize the same host, *Barbus trimaculatus,* throughout Africa; whereas *Neodiplozoon polycotyleus* infests several cyprinids even within the same area.

New Technique for Studying Canal Systems in Branchiura

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The use of this technique whereby coloured latex was injected into the genital ducts and alimentary canal of *Chonopeltis australis*, a member of the parasitic Branchiura, was found to be very successful during a detailed study of the morphology of these systems.

A glass needle with a point diameter of between 100 and 150 μm was drawn out of a micro-haematocrit centrifuge tube over a gas flame. The rear part of the needle was placed over the front portion of an ordinary steel needle, fixed and properly sealed with Pratley clear epoxy.

Boscotex latex was coloured with green Plaka, a water-soluble colouring matter used for colouring water-based paints. The coloured latex was diluted with 1 ml water per 5 ml latex. A disposable plastic syringe was used to inject the latex into the mouth or genital aperture of the parasite. Care was taken to inject just the right volume of latex to completely fill the systems without too much bulging. After the systems had been filled, the parasites were placed in a 15 – 20% acetic acid solution. The acid medium caused the latex to solidify. After 30 minutes, the parasites were transferred to 70% ethanol for further fixation. The material was cleared in lactophenol and mounted temporarily on hollow glass slides for further investigation.

Together with the use of histological reconstructions, this technique opens up a new field in the morphological studies of the digestive and reproductive systems of Branchiura.

Immunity to Canine Encephalitozoonosis

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Dosing experimental dogs with 10^6 spores of *Encephalitozoon cuniculi* grown in tissue culture produced infection but in no cases have clinical signs of encephalitozoonosis been observed [Bothma, Van Dellen & Stewart (1979). *J. S. Afr. Vet. Ass.* **50**, 135 – 144]. Even young puppies appear to be able to overcome experimental infection. In order to study the immunity developed against *Encephalitozoon,* an experimental adult female beagle dog was infected with 10^6 *Encephalitozoon cuniculi* spores grown in tissue culture. Leucocyte migration inhibition tests were carried out at regular intervals following infection. Similar tests were carried out in an uninfected control dog.

Blood was collected in heparin and leucocytes isolated on a Hypaque/Ficoll gradient washed twice and adjusted to 3×10^7 cells in RPMI 1640 medium with 10% foetal calf serum; 100 μl of antigen was added to 0.5 ml of cells (infected cells) and 100 μl medium added to 0.5 ml cells to act as a control. The antigen was prepared by sonication for 3 min of 10^7 *Encephalitozoon* spores grown in tissue cultue. Micro-capillary tubes were filled with cells, centrifuged at $900 \times g$ for 5 min, cut at the cell/medium interface and placed in migration chambers containing medium (three replicates of each). After 12 hours the area of migration was measured by projection of the outline on to white paper and drawing the outline. The area was measured with a planimeter. The area of migration of the infected cells was compared with the control cells.

Inhibition of migration was observed in the infected dog from 7 days after infection and persisted for 3 months. The uninfected control dog showed no inhibition of migration.

Epidemiological Survey of *Oestrus ovis* on a Farm in the Eastern Orange Free State

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Seventy-two lambs born in September 1982 ('pool lambs') were slaughtered in groups of six at monthly intervals. From the third month of the survey onwards, groups of three worm-free lambs ('monthly markers') were put out to pasture with the pool lambs.

In the pool lambs, *Oestrus ovis* was found in all groups from September 1982 when the lambs were three weeks old, to August 1983, when the lambs were 12 months old. Total numbers of *O. ovis* showed a peak in October 1982 and then declined very erratically until the end of the survey, with lowest numbers recorded in May 1983. First instar larvae were present in all groups in numbers ranging between 51 and 66% expressed as a percentage of the total population except during the period March to June when it was 68 – 88%. In the monthly markers, *O. ovis* was present from November 1982 (the first group) to April 1983 but from May to August very few larvae were found.

It is concluded that *O. ovis* infestation does not take place readily in winter (May to August) in this area and that the parasite overwinters as first instars in hosts infested before the month of May. Development of first instars is arrested from April to June but resumes during July.

From this it would appear that the ideal time for strategic chemical control of *O. ovis* in the eastern Orange Free State would be during May or June when new larval depositions have almost ceased and before development of arrested larvae is resumed in July.

Differences in Isoelectric Focusing Profiles of Proteins of *Schistosoma* species

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The soluble proteins in aqueous extracts of adults of *Schistosoma mansoni, S. rodhaini, S. haematobium, S. mattheei, S. margrebowiei* and *S. intercalatum* were separated by horizontal flat-bed isoelectric focusing (IEF) using the LKB Multiphore system. The polyacrylamide gel containing Ampholyte (pH 3.5 – 9.5) was prefocused for 10 min before the extracts were applied to filter-paper wicks placed 2 cm from the cathode. The samples were focused for 50 min at 5°C under a potential of 2000 V. The bands were stained with Coomassie Blue R250 and scanned with a Vitatron densitometer.

The patterns of the profiles differed with the species and between the sexes of the same species. Of the more than 30 bands, some were unique for the species although most bands were common to several species. No bands were common to all species. The patterns were sufficiently distinctive for them to be used to identify the species, unlike the patterns obtained with polyacrylamide gel electrophoresis (PAGE). However, it was not possible to group similar patterns to correspond with biological similarities as could be done when isoenzymes such as alpha naphthol acetate esterases were separated on PAGE. The advantage with IEF is that the proteins can be extracted from stored flukes that have lost enzyme activity on prolonged cold storage. It is, however, not as sensitive as the PAGE and IEF methods involving isoenzyme separations.

Scanning Electron Microscopy in the Identification of Strongyles and Atractids in the Equid

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The largest group of nematodes of equids, the Strongylidae, has 95 known species. In contrast, there are only two species of Atractidae, viz. *Crossocephalus viviparus* and *Probstmayria vivipara,* but they occur in millions in the zebra.

The identification of these species is based on the morphology of fine structures of the mouth (buccal) capsule, and of the genitalia, particularly of the male. A wide diversity of structures adorn the anterior and posterior extremities of nematodes. These include rows of petal-like structures and appendages of the male genital cone. There are also teeth in some species of varying sizes and shapes. The presence of teeth inside the buccal capsule of two *Strongylus* spp. was revealed with scanning electron microscopy. In *Strongylus equinus* a transverse section across the buccal capsule was made to remove the cuticle and the corona radiata, revealing the actual teeth at the base of the buccal capsule. This same method revealed the absence of teeth in *Strongylus ecientatus.*

The removal of the structures of the anterior extremity allows for the first time a clear examintion of the teech of *Strongylus,* a feature basic to the morphology of these nematodes, and could well be used to examine the teeth in other strongyles.

Modern Concepts of Diagnosis of Malaria

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The current resurgence of malaria is a source of grave concern. Consequently, the need for improved diagnostic tools for epidemiological studies and for control programmes has been increased. Antiplasmodial antibodies can be specifically detected by fluorescent tests, haemagglutination tests, immunoprecipitation techniques, RIA and ELISA. Some of these methods are useful and satisfactory for the detection of antibodies. However, antibodies are ubiquitous in residents of endemic areas and their presence is not a proof of current infection.

Generally, the diagnosis of malaria is based on microscopical identification of stained blood films. This technique is sensitive and the methodology does not require sophisticated equipment. The disadvantage is that it is not convenient for use on a large scale. One technician can accurately examine only 50 thick smears per day. A substitute for light microscopy is the fluorescent microscopic observation of Hoechst-stained DNA. This method is also time consuming and is useful only for research purposes.

It is for these reasons that experiments were performed to demonstrate malaria antigens by sensitive immunological methods. Antigens are detected by their ability to inhibit binding of antibodies to plastic microplates coated with different malaria preparations. The bound IgG is detected with radio-iodinated protein A or by addition of peroxidase-labelled reagent and substrate. The RIA could detect one parasitized erythrocyte (PE) per $10^5 - 10^6$ erythrocytes, while the ELISA could detect one per 10^4. Most tests are based on plasmodial preparations derived from *in vitro* cultures of *P. falciparum.* Therefore they are relatively expensive (US$5/sample). In addition, the source of antibodies for the tests is human immune serum, which is limited and not well defined.

Experiments are being carried out in an attempt to replace *P. falciparum* antigens with *P. berghei*-derived preparations and immune serum with a variety of monoclonal antibodies. Such a combination may be much cheaper and more suitable for mass diagnosis.

Another approach for detecting malaria parasites is based on DNA-DNA hybridization. Denatured and immobilized DNA fixed on nitrocellulose, hybridizes with radiolabelled *P. falciparum* DNA. The sensitivity of this assay is one PE per 10^6 erythrocytes and is much cheaper than RIA.

Some Observations on the Maintenance of *Schistosoma margrebowiei*

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Several laboratory-bred snail species, including *Bulinus* (*Physopsis*) sp. and *Bulinus depressus,* and an albino strain of *Bulinus tropicus,* were exposed to filtered lechwe and waterbuck faeces in August 1974. During the following October, a large number of these snails started shedding cercariae. *Saccostomus campestris* and *Praomys* (*Mastomys*) *coucha* were exposed to the cercariae, and 42 days later adult worms and ova were found in both rodent species that had been exposed to cercariae obtained from *B. tropicus* and *B. depressus.* These worms and ova were identified as *S. margrebowiei.* Fifty days after exposure to the cercariae obtained from *Physopsis,* adult worms and ova, identified as *S. leiperi,* were found in both *Mastomys* and *Saccostomus.*

The maintenance of *S. margrebowiei* has raised much concern over the past few years, as a very low percentage of exposed snails became infected. This problem has now reached the stage where the end of this laboratory strain of *S. margrebowiei* is in sight.

There was an infection rate of 50% in the snails that were exposed to the miracidia obtained from the natural hosts, the lechwe and the waterbuck, in 1974. This infection rate dropped gradually to 1% in the 16th generation.

In the first generation in *Mastomys* it was found that the worms died shortly after maturity, whilst in *Saccostomus* some worms were still alive after two years. However, in the 16th generation few worms reached maturity and died a short while later.

This poses a number of questions: (a) Is *B. tropicus* the wrong intermediate host? (b) Is *Saccostomus* a wrong laboratory host? (c) Is this schistosome strain weakened (unknowingly) by selection? (d) Is the same thing happening in all, or in some, of our other schistosome strains, perhaps at a much slower rate?

Efficiency and Safety of Fenthion-Methyl (Tiguvon®)* Application for Flea Control in Dogs

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Fleas (*Ctenocephalides canis* and *C. felis*) are periodic ectoparasites of major hygienic importance in cats and dogs, causing irritation, alopecia and, frequently, flea bite dermatitis [Kristensen (1978). *Tierarztl. Praxis* 6, 351 – 360]. Fleas act as biological vectors of the dog tapeworm, *Dipylidium caninum,* which may occasionally infect children.

Effective flea control is notoriously difficult although good results with a commercial formulation, Tiguvon Spotton® 20% m/v, have been published by small-animal practitioners [Lois (1978). *IV. Jornadas de Avepa, Tandil* 37 – 40; Carr (1980). *Can. Practice* 7, 69 and 72].

Further efficiency and safety tests called for exact dose volumes, and trials with artificial and naturally acquired flea infestations in dogs were carried out in order to make recommendations for use.

Fenthion-methyl has a systemic action and is rapidly absorbed through the skin, thus making it specifically advantageous for dermal applications. Residual efficiency was determined under controlled conditions by infesting a total of 42 dogs artificially with 80 – 100 fleas until a stable flea population was present. Following applications at dose rates of 5, 10 and 20 mg active substance per kilogram mass, dogs were re-infested six times at set intervals. Overall flea reduction was 78% for 5 mg/kg; 92.5% for 10 mg/kg and 93.5% for 20 mg/kg over a period of 21 days; thereafter residual efficiency gradually declined.

Trials with naturally acquired flea infestation were carried out with dogs kept in kennels in the Johannesburg area. The average pre-treatment flea count was 17, ranging from 6 to 50 fleas per dog.

Average flea counts on days 2, 7 and 14 after the first treatment were

0.6, 0.3 and 3.9 fleas per dog. At day 21 the flea population reached an average of 8.4 fleas per dog; however, no additional flea control (dusting or spraying) was employed in order to assure a continuously high flea pressure during the trial period (70 days). Applications were repeated at 14- or 21-day intervals. Residual efficiency conditions prevailing in Germany gave a 4-week protection after treatment with 8 – 10 mg/kg active substance [Schein and Hamel (1983). *Zbl. Bakt. Hyg.* **A256**, 257 – 272]. Flea populations on dogs were reduced 70 – 90% within one hour of treatment.

Particular attention was given to safety aspects following dermal applications, tested at increasing dose rates ranging from 5 to 200 mg/kg [Hopkins and Bladock (1984). *Vet. Med. Rev.* **2/1984**, 50 – 61]. Single dosages up to 80 mg/kg produced no signs of toxicity. Only at 160 and 200 mg/kg was typical organophosphate poisoning induced. Plasma cholinesterase remained in the normal range at 20 mg/kg; however, significant depression occurred at higher dose rates (40, 70 or 120 mg/kg). Plasma cholinesterase depression was most pronounced 4 to 6 days after treatment. During all regimens no adverse skin reactions were observed.

Simultaneous application of Fenthion-methyl at dose rates of 11.6 – 18.8 mg/kg, and Propoxur or Dichlorvos collars or Bacdip wash treatment at 1 000 ppm, were well tolerated by dogs.

We conclude that Fenthion-methyl is an efficient and safe product for flea control in dogs.

*Registered trademark of Bayer A.G., Leverkusen.

Cutaneous Nodules of *Besnoitia* sp. in a Feral Rodent

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Two species of *Besnoitia* have so far been described from South Africa: *B. besnoiti* from cattle, impala, blue wildebeest and kudu [Hofmeyer (1945). *J. S. Afr. Vet. Med. Ass.* **16**, 102 – 109. Pols (1960). *Onderstepoort J. Vet. Res.* **28**, 25 – 356. Basson (1965). *J. S. Afr. Vet. Ass.* **36**, 578] and *B. bennetti* from horses, a mule and donkeys [Bigalke (1970). *J. Parasitol.* **56**, (4, section II 29]. No besnoitial infections have so far been described from rodents in Africa, although such infections have been reported from the USA, Peru and Khazakhstan in Central Asia.

This presentation is a report of besnoitial nodules found on both ears of a bushveld gerbil *Tatera leucogaster*, caught at Rundu in Namibia. Sections showed a nodule packed with cystozoites of *Besnoitia*. There was no infiltration either around or within the cyst and there was no trabeculation within it. Cystozoites undergoing endodyogeny were common.

Further study will aim at defining the species of *Besnoitia* found and in determining its possible connection to, and importance in, the epizootiology of besnoitiosis of domesticated cattle and/or antelopes.

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Embryonic Development of *Schistosoma mansoni* and *S. haematobium*: Egg Envelope Formation

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The origin and fine structure of protective envelopes of the schistosome egg have been studied by means of electron microscopy and histochemistry. Eggs of *S. mansoni* and *S. haematobium*, representing consecutive stages of development, have been reconstructed from serial thin and semi-thin sections. The embryonic development of schistosome eggs takes place during their migration through the mammalian host tissue. Cleavage is total and unequal, resulting in the formation of three types of blastomeres: macro-, meso- and micromeres. The future miracidium differentiates only from mesomeres and micromeres, while macromeres and some mesomeres participate in the formation of egg envelopes. The three main envelopes, the egg-shell, and the outer and inner envelopes are formed around the developing embryos.

The egg-shell is formed in the ootype from shell-globule material of vitelline cells, and encloses the fertilized oocyte and numerous vitelline cells.

The outer envelope is formed by macromeres. In both species examined, two macromeres detach from the embryo and their cytoplasm fuses together forming a syncytial outer envelope. The macromere nuclei, which contain prominent nucleoli, progressively become irregular in shape. They usually persist in this layer in the early embryos but disappear completely in the advanced stages of embryonic development. The cytoplasm of this envelope contains, in an early stage, several mitochondria and lipid droplets, and a high concentration of free ribosomes. In the advanced stages it becomes an anucleate, granular layer very rich in free ribosomes.

The inner envelope is formed by several mesomeres which become detached from the surface of the developing embryo after outer envelope formation. The cytoplasm of mesomeres after their fusion also forms a syncytial layer surrounding the embryo beneath the outer envelope. In advanced stages of embryogenesis, the inner envelope (6 – 9 μm thick) is characterized by the presence of large, flattened nuclei with prominent, spherical nucleoli and dense chromatin islands. The cytoplasm contains a well-developed granular endoplasmic reticulum, numerous large lipid droplets of chemically saturated type and extended aggregations of α-glycogen rosettes. As indicated by the ultrastructure of the nuclei and cytoplasm, the inner envelope has features of an energy storage (glycogen, lipids), metabolically active syncytial layer.

The large, acid phosphatase-positive areas of focal cytoplasmic degradation which appear in egg envelopes just before the hatching of miracidia seem to be involved in the autolysis process. It appears that the structural components of the envelope and nutritive reserves are re-absorbed by miracidia before hatching.

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Anthemosoma sp. Isolated from *Aethomys namaquensis* in Namibia

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An adult *Aethomys namaquensis*, caught on the bank of the Löwen river some 90 km south of Keetmanshoop in Namibia, was found at splenectomy to have an enormously enlarged spleen. Thin blood smears taken daily after the operation showed intra-erythrocytic parasites ranging from young trophozoites, through immature schizonts to mature ones containing 8 to 16 merozoites (on one occasion 24 merozoites were counted in a single schizont). Forms suggesting immature gametocytes were also seen. The mature merozoites were never round but always pyriform — suggesting piroplasms. Malarial pigment was never seen in any of the above forms.

The parasite was diagnosed as being an *Athemosoma* sp. which morphologically closely resembled *A. garnhami* previously described in 1969 by Landau *et al.* [(1969). *C. R. Acad. Sci. Paris* **268** (Série D), 873 – 875] as a new genus and a new species from *Acomys percivali* in Ethiopia. Later (1981) Levine created a new family, Anthemosomatidae, to accommodate the apicomplexan genus *Anthemosoma* and placed it in the order Piroplasmorida Wenyon, 1926 [Levine (1981). *J. Parasitol.* **67**, 440 – 441]. This is the second report of an *Anthemosoma* sp. isolated from a mammal.

However, as there are distinct differences between *A. garnhami* and the present isolate with regard to the spectrum of infectivity to laboratory animals, it is not yet clear whether the parasite isolated from *Aethomys* constitutes a different strain of *Anthemosoma garnhami*, or is a new species.

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