THE PARASITOLOGICAL SOCIETY OF SOUTHERN AFRICA

The following are abstracts of papers presented at the Annual Scientific Meeting on 23-26 June 1992. The congress was held at the Loskop Dam and the theme was "Parasitology into the 21st Century".

DIE PARASITOLOGIESE VERENIGING VAN SUIDELIKE AFRIKA

Die volgende is uittreksels van referate wat gedurende die Jaarlikse Wetenskaplike Vergadering op 23-26 Junie 1992 gelewer is. Die kongres was by Loskopdam en die tema was "Parasitology into the 21st Century".

Six different species of *Theileria* can be diagnosed using specific DNA probes

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The complete small subunit ribosomal RNA (srRNA) genes of *Theileria parva* and *Theileria buffeli* Marula were cloned and sequenced. Two primers were designed which permitted the specific amplification of a part of the *Theileria* srRNA gene from *Theileria*-infected cell samples which were predominantly (> 99 %) bovine DNA. Partial srRNA genes for *Theileria annulata, Theileria taurotragi, Theileria mutans* and an unidentified parasite referred to as *Theileria* sp. (buffalo) were amplified using these primers, cloned and sequenced. An alignment of the sequences was generated from which 6 oligonucleotide probes, corresponding to species-specific regions, were designed.

These probes were demonstrated to provide unequivocal +/- identification of each of the 6 species either by direct detection of parasite srRNA or by hybridization to PCR-amplified parasite srRNA genes. The probes did not distinguish *T. p. lawrencei*, the Corridor disease-producing parasite, from *T. p. parva* but did distinguish between *T. buffeli* and *Theileria sergenti* which were previously believed to be synonyms for one species. The identity of *Theileria* sp. (buffalo) remained uncertain.

The use of the probes during a Corridor disease outbreak in the northern Transvaal shows how important it is, during an epidemic, to be able to distinguish between the various species of *Theileria* with widely differing pathogenicities.

Piroplasm evolution and the taxonomic status of *Babesia equi*

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Within the last 20 years, evolutionary studies have been revolutionised by the widespread application of molecular biological techniques, particularly those generating nucleic acid sequence information. Many taxonomic problems, previously insoluble by classical means, are being resolved by the addition of sequence comparison information to the body of knowledge already extant. A molecule particularly suitable for phylogenetic studies is that of the small subunit ribosomal RNA (srRNA) gene. Ribosomes are universally present in all cells and their functional similarities are reflected in regions of conserved nucleic acid sequence within the gene, interspersed with often highly variable regions which define the secondary structure of the molecule. The average length of an eukaryotic srRNA molecule is about 1 700 base pairs, short enough to allow easy sequencing but long enough to provide meaningful phylogenetic information.

The relationship between *Babesia equi, Babesia caballi* and the *Theileria* parasites has long been in dispute and we have used the ribosomal RNA approach in an attempt to resolve this. We have amplified, cloned and sequenced the srRNA genes of *B. equi, B. caballi* and 2 *Theileria* species and have compared these with a panel of previously published srRNA gene sequences. These include 4 other Apicomplexan species, and phylogenetic trees have been derived using computer programmes based on different algorithms.

Our results indicate unequivocally that *B.equi,* while showing certain sequence similarities to the babesias, bears considerable resemblance to the theilerias. It suggests that *B. equi* has evolved from an ancestral intermediate between the genera *Theileria* and *Babesia.*

The male reproductive system in *Argulus japonicus* (Crustacea: Branchiura)

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In spite of numerous investigations into sperm transfer in *Argulus* it still remains an enigma. The histomorphology of the male reproductive system and surface morphology of the accessory sexual structures in *Argulus japonicus* are described from serial sagittal and transverse sections as well as scanning electron micrographs.

The male reproductive system consists of 2 testes and 2 vasa efferentia uniting to form a single vesicula seminalis which divides into 2 vasa deferentia. Two prostate complexes situated in the carapacial haemocoel drain into respective reservoirs and lead to efferent ducts. The latter unite with the deferent ducts from the vesicula seminalis to form the ejaculatory ducts which are guarded by sphincters. The lumens of the ejaculatory ducts are not connected to the cuticle-lined genital atrium but are separated by fibrous cells.

Accessory sexual structures consist of a peg and socket respectively on the third and fourth appendages on both sides. Cross-sections reveal that the socket extends into a haemocoel in the coxapodite of the third leg. A striated muscle fibre extends from the deepest end of the socket towards an apodeme on the anterior cuticle and suggests that the size of the socket can be altered at will. Cross-sections of the peg show nervous tissue suggesting that this structure has a sensory function.

Males of *A. japonicus* produce a sperm packet consisting of sperm held together by ejaculate from the prostate gland. The presence of the latter suggests that the sperm packet will either be transferred to females in a manner similar to the spermatophores in *Dolops ranarum* or that the sperm packet is deposited in the socket situated on the third thoracic appendage of males which will act as a semen capsule as was suggested by eighteenth century workers.

Global distribution of *Achatina* tagged by parasites

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A previous report of trichodinid parasites associated with the giant African snail *Achatina* in Mauritius indicated that only 1 trichodinid species was associated with both *Achatina fulica* and *Achatina panthera*. A subsequent investigation into the morphology as well as detailed statistical analyses revealed that 2 parasite species were in fact present on the achatinids of Mauritius representing a new genus with 2 new species, i.e. *Pallitrichodina rogenae* Van As & Basson (in press) and *Pallitrichodina stephani* Van As & Basson (in press), and occurring on both snail species.

During a research visit to Taiwan, specimens of *A. fulica* were found to be distributed on the island. This species harboured a single trichodinid species in the mantle cavity, i.e. *P. rogenae*.

Although achatinids are endemic to Africa, they are widespread in various parts of the world today. A clear trail of distribution from Mauritius can be followed in an easterly direction. According to the literature 2 possible routes of distribution from Mauritius to Malaya and Ceylon exist, either via India or direetly from Mauritius. The occurrence of the trichodinid species was used to illustrate that the introduction of *A. fulica* into Malaya and Ceylon was probably via India. From our data certain deductions were made concerning the host/parasite relationships of achatinids in the endemic distribution areas of East Africa.

Future challenges facing animal parasitology in southern Africa and some possible strategies to accommodate them

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Animal parasitology operates in a considerably more complex environment than many other scientific disciplines. Cognisance must inevitably always be taken of factors which affect the host as well as the parasite.

If the human population explosion continues, it is expected that considerably more food of animal origin will be required. This means that either greater numbers of animals will have to be produced or an increased production per head will be necessary. Both options have implications for parasitology. For example: more efficient utilisation of tsetse-infested land in Africa in general; greater intensification, which usually favours numbers, higher production per head implies inter alia the need to decrease parasite-induced losses, which are still unacceptably high.

"Green" and related issues are likely to become even more relevant, although they will probably be ameliorated by some degree of political tolerance of the almost inevitable environmental consequences of the anticipated population growth (AIDS may alter this prediction).

Animal parasitologists will have to develop alternatives for the use of "hardcore" polluting or residue - forming chemicals applied topically or systemically as parasiticides and therapeutics. Integrated control methods, incorporating sound management principles; genetically engineered, environmentally friendly products; the development of easy care animals by conventional and especially biotechnological means, all offer exciting prospects.

The anticipated global change may present its own indirect challenges such as changing ecological conditions, and thus the associated parasitological fauna.

Parasitologically-oriented biotechnology is bound to blossom in years to come. Anticipated benefits should be in the field of molecular biology, genetically engineered vaccines, diagnostic probes and, especially, transgenic animals, as outlined below.

Parasitologists should, in their own interest, help to address the growing problem of decreased profitability of livestock farming. Easy care animals offer considerable reward, also in this respect, in a continent blessed with numerous breeds or types of adapted stock and wild animal species. Purposeful selection, gene transfer, immunisation with "new generation vaccines" and sound management practices aimed at avoiding excessive exposure to parasites and stress, come to mind.

The escalating problem of the development of resistance against chemical insecticides, worm remedies and drugs by external, internal and unicellular parasites should be regarded as excellent opportunities to discover alternative control methods, using the technologies referred to above and others awaiting discovery by creative thinkers.

Parasitologists should not, however, be altogether blinded by the glare of new technologies and forget about the importance of classical biological studies aimed inter alia at elucidating life cycles of parasites, studying the epidemiology and immunology of infections or infestations, and the value of classifying organisms. A lot remains to be done in these fields and they form the foundations on which the futuristic technologies are being built. Computer-based technology, which includes simulation modelling, has become an indispensable partner in studies on most of the abovementioned disciplines and has created challenging new opportunities and approaches.

Reversal of choloroquine resistance in *Plasmodium falciparum* by the calcium channel blocker, flunarizine

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Chloroquine resistance is now a major health problem in areas where malaria is endemic. Recent evidence has revealed that the calcium channel blocker, verapamil, reversed the chloroquine resistance of *Plasmodium falciparum* chloroquine-resistant strain 3 (FCR-3) to full chloroquine sensitivity.

We investigated the effect of a new calcium channel blocker, flunarizine, which has minimal cardiac effects, on the reversal of chloroquine resistance in FCR-3.

This study was carried out on an in vitro culture of FCR-3 maintained in human O-positive blood plus RPMI 1640 medium. The parasites were synchronised to an early ring stage and diluted to 0,5% parasitaemia and 1% haematocrit. Drug sensitivity in 96-well microtitre plates was assessed using the tritiated hypoxanthine uptake method to measure parasite growth. Inhibitory concentrations of 50% (IC_{50}) were calculated for predetermined fixed ratios of chloroquine and flunarizine. Isobolograms were constructed in order to determine possible additive or synergistic effects of chloroquine and flunarizine.

The IC_{50} for chloroquine was found to be $3{,}46 \times 10^{-7}$M, while that of flunarizine was $1{,}22 \times 10^{-5}$M. Thus flunarizine by itself has antimalarial activity, although this is in the order of 200 times lower than that of chloroquine. Flunarizine potentiated the action of chloroquine, so that the IC_{50} of chloroquine in the presence of 4×10^{-6} M flunarizine was reduced to $8{,}4 \times 10^{-8}$ M. Analysis of the isobologram revealed that the combination of chloroquine and flunarizine were at least additive, if not synergistic.

This combinantion may therefore have therapeutic potential in the treatment of chloroquine-resistant malaria.

In vitro techniques for detecting ivermectin resistance in *Haemonchus contortus*

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The indiscriminate repeated use of ivermectin on some farms in the Republic of South Africa has resulted in the emergence of ivermectin resistant field strains of *Haemonchus contortus*. Evidence of reduced efficacy of ivermectin to control *H. contortus* gives rise to the need for a rapid and cost effective in vitro test to detect such strains. Infective third-stage larvae of *H. contortus* known to be resistant to ivermectin were placed in various concentrations of the drug for varying times. Subsequently their ability to migrate was compared with that of similarly treated larvae of strains known to be susceptible to ivermectin. The criterion used to determine resistance was their ability to migrate through either a 38 µm aperture sieve or out of gelled agar after exposure to ivermectin.

The results obtained by these 2 techniques agreed with those obtained with critical controlled tests, thereby confirming that in vitro techniques may be used to identify resistant strains.

How *Haemoproteus columbae* causes mortality in doves

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Haemoproteus columbae is a common blood parasite of members of the dove family, the Columbidae. This parasite is considered to be benign under natural conditions. However, mortalities can occur under intensive conditions such as in aviaries where the pigeon louse fly *Pseudolynchia canariensis*, which acts as vector, might be common, causing abnormally high *H. columbae* infections.

A life cycle in the tissues of the dove is proposed where sporozoites which enter the bloodstream form first generation schizonts in the endothelial cells of the bloodvessels. The merozoites which are released from these schizonts form second generation uni- or multilocular schizonts within muscle cells. When the merozoites are released from these second generation schizonts extensive muscle necrosis results and the release of toxic materials during this process could account for mortality.

Previous reports suggested that "aberrant" *Leucocytozoon*-type organisms caused mortality in aviary birds. We now believe that at least some of those mortalities were caused by heavy infections of *Haemoproteus*.

Intestinal helminths of the Bushmen of northern Namibia

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Results of surveys conducted between February 1986 and April 1989, involving 348 subjects in Bushmanland and families in Kaudom Game Reserve, showed a high prevalence of hookworm (63-100%), *Strongyloides stercoralis* (25%) and *Trichuris* (KGR) (35%). There were some spurious *Physaloptera* sp. infections and several Bushmen had thin-shelled, hookworm-like eggs with longitudinally folded embryos in their faeces. Chinese hamsters (*Cricetulus griseus*) which were fed third-stage hookworm larvae (L_3) per os and killed >60 d later, harboured adults of *Necator americanus*. In March 1990, stool cultures examined 24 - 72 h later revealed free-living adult *Strongyloides fülleborni*, together with rhabditoid larvae of *S. stercoralis*.

In March 1990, the translocation of 4 000 men, women and children of Bushman descent, mainly from West Caprivi and western Bushmanland, to Schmidtsdrift (SA), posed the problem of possible introduction of new parasites into the area. Two surveys were conducted 8 months apart, the first 4 months and the second a year after the Bushmen reached SA. A total of 53 and 140 stools, respectively, were collected from children aged 6-16 years. Two grams of each stool were prepared by the formol-ether method, and the eggs were quantified/gram. At 4 months, 48/53(91 %) had hookworm, 42(79 %) *S. fülleborni*, 25(47%) *S. stercoralis*, 9(7%) *Trichuris*, 1(2%) *Taenia* sp. and 24/42(57%) harboured both strongylid species. Eight months later, 112/140(80%) had hookworm, 57(41) *S. fülleborni*, 17(12%) *S. stercoralis*, 24(17%) *Trichuris*, 2(<2%) *Ascaris*, 1(<1%) *Taenia* sp., 1(<1%) *Hymenolepis nana*, and 13/57(23%) were infected with both strongylids. Faecal egg counts ranged from 2 to 3870 (hookworm), 2 to 2988 *S. fülleborni* and larval counts for *S. stercoralis*, 3 to 1512. Both *Ascaris* infections were presumably contracted locally.

Morphology of the reproductive systems of *Chonopeltis victori* Avenant Oldewage, 1991 (Crustacea: Branchiura)

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Although 12 species of the piscine ectoparasitic genus *Chonopeltis* have hitherto been described, morphological and anatomical information on the internal organs especially are scant. The majority of papers on *Chonopeltis* spp. are based typically on whole mount studies with little, if any information on the internal structures.

The present study intends to provide a detailed morphological description of the reproductive systems of *Chonopeltis victori*, using histological serial sections and graphic reconstructions.

The male reproductive system consists of paired testes, vasa efferentia, vasa deferentia, ductus ejaculatoria, elongate prostate glands, a visicula seminalis and a genital atrium whilst the female reproductive system consists of a single ovary, 2 oviducts (of which only one is functional) a genital atrium as well as paired spermathecae and spermathecal ducts. In both sexes, several distinct secondary sexual characters are prominent externally.

Extensive and detailed morphological knowledge of all aspects of these parasites is a prerequisite for understanding the adaptations needed for a piscine ectoparasitic existence.

Tick taxonomy: beyond 2000 (from naked eye to nucleotides)

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Major changes in the concepts and techniques used in tick taxonomy from the earliest times to the present are reviewed. Future trends in tick taxonomy are outlined.

Humans have probably been aware of ticks since their earliest origins when they came across these parasites while foraging. Certainly, ticks must have been a major nuisance when animals were first domesticated (+/-8000 BC). Though ticks were mentioned by both Aristotle (384-322 BC) and Pliny (AD 77), it was Linnaeus (1758) who described the first tick species.

The earliest tick taxonomists had access to only the simplest optical equipment for their examinations (perhaps the naked eye). The typological concept of species which they used dated back to Aristotle and Plato and the characters used to define and classify species were mainly morphological.

When optical equipment improved, in the early and middle 20th century, taxonomists were able to view their specimens better and therefore use subtler morphological characters. Species were now viewed as "*...groups of interbreeding natural populations...*". Behaviour, ecology, physiology etc. thus became more relevant as taxonomic tools.

The advent of the electron microscope, molecular and computer revolutions and cladistic analysis have transformed tick taxonomy and will continue transforming it well into the 21st century.

The electron microscope allows ticks to be examined more clearly.

Protein electrophoresis enables the separation of sibling species. The study of immune reactions and the examination of nuclear and mitochondrial DNA provides an additional new set of characters for the taxonomist.

Computers enable sophisticated statistical analyses to be made in order to separate close species. Image analysis will alleviate the tedium of making hundreds of routine measurements on specimens. Computer catalogues of tick collections allow the rapid location of specimens, relevant literature and the production of distribution maps of species.

Finally, cladistics promises an objective means of classifying and determining the evolutionary history of ticks.

Chlamydia-like inclusions in red blood cells of turkeys and francolins

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During 1990-1991 small (0,1 to 0,2 m) inclusions were found, often paired, in blood smears of turkeys kept in the Institute's insect proof isolation unit, which was used for blood parasite transmission.

The organisms spread throughout the turkey colony in the absence of biting insects and without syringe passage, reaching high counts in some birds and maintaining detectable infection for long periods.

Transmission electron microscopy revealed single organisms which appeared similar morphologically and in size to large reticulate and intermediate bodies in the early and middle stages of *Chlamydia* without, however, forming colonies. The fact that these organisms were able to spread from turkey to turkey without the help of biting arthropods, precludes them from being Rickettsiae.

The same intra-erythrocytic organisms were found in blood smears of Swainson's francolins. Francolin and guineafowl blood from different areas, which had been used in subinoculation trials in the isolation unit, was probably the source of infection of the turkeys.

Travel-related parasitic hazards

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Although still largely unknown in South Africa, Travel Medicine is a rapidly growing medical speciality in its own right. This is not surprising considering that, worldwide, some 500 million tourist arrivals, equivalent to about 10% of the total world population, are recorded annually. Tourists are made increasingly aware of the various travel-associated health risks, in which various infections play a major role. A Travel Health Information Centre, the first in this country, was therefore recently established in Johannesburg, primarily as a source of reference for the medical and allied professions.

Parasites are common mementoes brought home from otherwise happy holidays or successful business trips. Among the more common parasitic infections acquired while travelling are malaria, giardiasis and amoebiasis.

Malaria, more than any other health hazard, is a matter of great concern to travellers. It is also the most difficult infection to prevent effectively and safely in all travellers. Giardiasis and amoebiasis, important causes of travellers' diarrhoea, may be easier to prevent by the observation of a few simple precautions.

Less common parasitic infections acquired by travellers include African trypanosomiasis, leishmaniasis and cryptosporidiosis.

One of the problems facing the traveller who becomes ill shortly after his return home, is the unfamiliarity of his/her hometown medical practitioner with exotic infections and their clinical manifestations. This tends to result in extended and more serious illness, a higher incidence of complications and, occasionally, fatalities which might have been avoided. It is therefore important for medical practitioners to obtain a proper travel history from their patients and, if necessary, to refer them timeously to an appropriate centre of expertise in exotic infections.

A parasitological survey of Bushmen (SAN) immigrants from Namibia

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During 1990 a group of approximately 4 000 Bushmen immigrants from Namibia were relocated at Schmidtsdrift near Kimberley. The group comprised men, women and children originally from Bushmanland, the western Caprivi and southern Angola. Most had spent some time at the Omega base in western Caprivi. Uneasiness on the part of the local farming community regarding the potential introduction of exotic diseases mandated that a survey for parasitoses be carried out, even if only a partial one. The survey encompassed a search for serum antibodies against hepatitis B and toxoplasmosis as well as the occurrence of lymphatic filariasis, intestinal parasitoses, and, only partially, malaria and leishmaniases. Only the parasitoses will be reported here. A total of 371 soldiers (who had all served north of the Border) showed no microfilariae of *Wucheria bancrofti* in thick blood smears taken between 22:00 and 02:00, but an elevated eosinophilia was found in 281 (75.4%).

Stool examinations of 193 children (6 - 16 yr) were positive for hookworm, *Strongyloides fülleborni, Strongyloides stercoralis, Trichuris trichiura, Ascaris, Taenia* spp. and *Hymenolepis nana.* One hundred and forty stools were also examined for coprozoic protozoa. *S. fülleborni* was confirmed for the first time in southern Africa as a human pathogen existing side by side with *S. stercoralis.* Malaria had been treated as it occurred. At the time of the survey only one case of *Plasmodium ovale* infection was seen. Two unequivocal cases of cutaneous leishmaniasis were diagnosed clinically (both in the healing stage).

In conclusion it could be stated that the immigrant Bushmen did not constitute a reservoir for the introduction of exotic diseases to the northern Cape (even though no information regarding potential vectors of malaria and leishmaniasis is on hand).

The efficacy of an albendazole intra-ruminal slow-release capsule against nematode parasites in sheep and on the infectivity of a pasture

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Albendazole intra-ruminal slow-release capsules (SRC) administered to sheep grazing on irrigated pastures in the southern Cape Province, controlled *Trichostrongylus* for 91 days and *Haemonchus* and *Teladorsagia* for 61 days. Treating sheep with the SRC at 47 - 61 day intervals over a period of 10 months, significantly ($P < 0,05$) reduced the infective potential of the pasture. Compared with sheep which received 2 disophenol and 3 broad spectrum anthelmintic treatments during the same period, the SRC reduced the infective potential of the pasture by 71,7%, while weekly drenching with albendazole over the same period, resulted in a significant ($P < 0,05$) increase of 73,4 % in the infective potential of the pasture.

Rhipicephalus simus as transovarial vector of *Babesia trautmanni*

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Rhipicephalus simus was, for the first time, proven to be a transovarial vector of *Babesia trautmanni* of domestic pigs. *R. simus* adult ticks were fed on a *B. trautmanni* reacting splenectomized pig. Haemolymph smears of experimentally infected female ticks were examined 5-17 days after engorgement and 50% were positive for *B. trautmanni* kinetes. The larval stage was fed on rabbits and the resulting nymphs were either fed on a rabbit or a susceptible splenectomized pig. The ensuing adults were again fed on a susceptible splenectomized pig. Adult ticks, fed on rabbits during the larval and nymphal stages, were also fed on a susceptible splenectomized pig. The features of the infection in these pigs included a prepatent period of 6-8 days post-tick infestation, a febrile reaction for 1-3 days and a maximum parasitaemia of 0,2%-2%. Other clinical signs were mild inappetence and listlessness. The 3 pigs recovered without any treatment.

Frequency of diminazene-resistant trypanosomes in populations of *Trypanosoma congolense* arising in infected animals following treatment with diminazene

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Diminazene aceturate (Berenil(R), Veriben(R)) is commonly used as a therapeutic agent for the treatment of trypanosomiasis in African domestic livestock. Resistance to the compound has been described in many sites across Africa. However, the factors contributing to this resistance phenotype are not known. In the work described here the diminazene-resistance phenotype of trypanosomes refractory to treatment with diminazene aceturate was determined in vivo.

Ten mice were infected with *Trypanosoma congolense* IL 3274. After detection of parasitaemia, animals were treated with diminazene aceturate at a dose of 25 mg kg^{-1} body mass. After a period of aparasitaemia, trypanosomes reappeared in all animals. At this stage the mice were exsanguinated, the level of parasitaemia quantified and the blood diluted in phosphate saline glucose, to prepare 5 different trypanosome inoculum sizes in 0,2 ml (10^2, 10^3, 10^4, 10^5 and 10^6). Each inoculum size was administered intravenously to 100 mice. Immediately after challenge, 50 mice in each group were intraperitoneally injected with diminazene aceturate at a dose of 25 mg kg^{-1} body mass; the other 50 mice received 0,2 ml distilled water, intraperitoneally, as an infectivity control. Development of parasitaemia was monitored for 100 days. All infectivity controls became parasitaemic. In contrast, in the groups of mice inoculated with 10^2, 10^3, 10^4, 10^5 or 10^6 trypanosomes and treated with diminazene aceturate, 4/50, 11/50, 13/50, 28/50 and 39/50 mice were detected parasitaemic, respectively. Analysis of the data indicated that, with an inoculum of 10^2 trypanosomes, 0,001% of the population was resistant to the drug dosage used. This decreased to 0,00015 % of the population with an inoculum of 106 trypanosomes. The data could be modelled using logistic regression and indicated that, in trypanosome populations relapsing following treatment with diminazene, the proportion of trypanosomes sensitive to diminazene was inversely related to the population size and was in excess of 99,9%.

First description of the male of *Dinemoura latifolia* Steenstrup & Lütken, 1861 (Copepoda: Pandaridae)

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Of the 4 known species of the genus *Dinemoura* Latreille, 1829, only 1 has been reported on sharks off the coast of Africa. *Dinemoura latifolia* has been recorded from *Prionace glauca, Mola mola* and *Isurus glaucus* at Cape Point and in Baja Forta, Angola. We recently had the opportunity to examine samples from Cape Recife on the south-eastern shores of South Africa and found a number of males of *D. latifolia* on *Isurus oxyrhinchus*. The hosts ranged from 1 600-2 100 mm in length. Attachment was to the pelvic fins except for a single occurrence of 7 parasites behind the teeth. A number of *Pandarus* sp. were found on the skin of this particular host. This is in agreement with the suggestion that *Dinemoura* and *Pandarus* compete for a similar niche on single hosts and that *Pandarus* is the dominant species.

Previous descriptions of the male are incomplete and differ from the present study in a number of aspects. The frontal plates are not centrally indented, but tapered and the first thoracic segment is not extended posteriorly. The same applies to the second and third thoracic segments. The genital segment is square and not rounded, as reported before. The lack of modifications to the third swimming leg negates a previous suggestion that a modified third leg is characteristic of the male of this species.

Fluorescence analysis of the interaction of isometamidium (Samorin(R)) with *Trypanosoma congolense*

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Chemotherapy of trypanosomiasis in cattle, sheep and goats is currently dependent upon the salts of 3 compounds; the aromatic diamidine diminazene, the phenanthridine homidium and the phenanthridine-aromatic diamidine isometamidium. Of these compounds, isometamidium (Samorin(R), Trypamidium(R)) is the only one routinely used as a prophylactic agent.

Although isometamidium has been used in the field for over 30 years very little is known about its mode of action. In the work described here the fluorescence property of isometamidium was used to examine the interaction of the molecule with an isometamidium-sensitive clone of *Trypanosoma congolense*, using quantitative fluorescence measurements. In vitro-derived bloodstream forms of *T. congolense* IL 1180 were incubated in medium containing isometamidium chloride at a concentration of either 50 or 100 mg ml^{-1} and analysed in an SLM Aminco 8000™ fluorometer with an excitation wavelength of 374 nm. Interaction of isometamidium with *T. congolense* IL 1180 at 37°C for 180 minutes resulted in a gradual alteration of the λmax from that of the free compound (600 nm) to that of bound isometamidium (584 nm). Furthermore, a concomitant increase in fluorescence intensity of approximately two-fold was observed. This alteration in fluorescence was temperature dependent and was inhibited by the addition of N-ethylmaleimide. Finally, addition of digitonin to medium containing trypanosomes and isometamidium resulted in a rapid increase in fluorescence intensity of approximately 4 times that observed with intact cells.

The data indicated that the observed alteration in fluorescence was due to interaction of isometamidium with an intracellular component(s), that isometamidium is transported across the plasma membrane and that the fluorescence properties of isometamidium enable it to be used as a probe to characterise the interaction of the molecule with *T. congolense.*

Laboratory diagnosis of heartworm (*Dirofilaria immitis*) infection in the dog

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The diagnosis of heartworm infection in dogs is usually based on the demonstration of microfilariae in the peripheral blood, using the modified Knott's test or membrane filtration. Care must be taken to differentiate the microfilariae of *Dirofilaria immitis* from those of the nonpathogenic *Dirofilaria repens* as well as *Dipetalonema* spp., namely *Dipetalonema reconditum* and *Dipetalonema dracunculoides*. Acid phosphatase staining is the technique of choice for such differentiation. Suspected occult infections can be determined by means of various commercially available ELISA test kits for heartworm antigen. Clinical signs, electrocardiography, clinical pathology and especially radiography can be useful adjuncts in the diagnosis. In particular, enlargement of the caudal lobar arteries and their branches, as revealed by thoracic radiographs, is regarded as pathognomonic for heartworm disease.

Cross-resistance between avermectins and milbemycins: Oral activity of ivermectin and moxidectin against ivermectin-resistant and susceptible nematodes

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In order to determine whether there is cross-resistance between avermectins and milbemycin anthelmintics, ivermectin and moxidectin sheep drenches were tested against ivermectin-resistant and susceptible isolates of *Ostertagia circumcincta* and *Trichostrongylus colubriformis* in sheep. None of the isolates had been exposed to moxidectin treatment previously. Worm counts following necropsy showed the dosage limiting species of the susceptible isolates to be *T. colubriformis* for both compounds whereas the dosage limiting species among the resistant isolates was *0. circumcincta* for both compounds. Ivermectin dosages required to remove 95 % of the resistant *0. circumcincta* and *T. colubriformis* were 23x and 6x higher than the dosages required to remove the same percentage of susceptible isolates, respectively. Moxidectin dosages required to remove 95% of the resistant *0. circumcincta* and *T. colubriformis* were 31x and 9x higher, respectively, than the dosages required to remove the same percentage of susceptible isolates. It is concluded that ivermectin and moxidectin share a similar mode of action which is reflected in cross-resistance between the 2 compounds.

Paraherquamide-overview of safety and efficacy

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Paraherquamide, an oxindole alkaloid metabolite of *Penicillium paraherquei* and *Penicillium citrinum*, is a new anthelmintic entity with a novel mode of action. It was tested against adult stages of 6 gastrointestinal nematodes of sheep at single, oral dosages of 0,25, 0,5, 1,0 or 2,0 mg kg^{-1}. At dosages ≥0,5 mg kg^{-1} there was ≥95% removal of *Haemonchus contortus*, *Ostertagia circumcincta*, *Trichostrongylus axei*, *Trichostrongylus colubriformis* and *Cooperia curticei*. The isolate of *H. contortus* used was ivermectin-resistant and the isolate of *T. colubriformis* used was benzimidazole- and ivermectin-resistant. No adverse reaction was observed in the sheep. Paraherquamide was also tested against the adult stages of 9 gastrointestinal and lung nematodes of calves at single, oral dosages of 0,5, 1,0, 2,0 or 4,0 mg kg^{-1}. At dosages ≥1,0 mg kg^{-1} there was ≥95% removal of *Haemonchus placei*, *Ostertagia ostertagi*, *T. axei*, *T. colubriformis*, *C. oncophora*, *Nematodirus helvetianus*, *Oesophagostomum radiatum* and *Dictyocaulus viviparus*. No adverse reaction was observed in the calves. Paraherquamide was subsequently tested against the adult stages of 5 intestinal nematodes of dogs at single, oral dosages of 0,5, 1,0 or 2,0 mg kg^{-1}. No dosage produced useful broad-spectrum activity against *Ancylostoma caninum*, *Uncinaria stenocephala*, *Toxascaris leonina*, *Trichuris vulpis* or *Strongyloides stercoralis*. All dosages, however, did produce significant toxicosis. The mode of action of paraherquamide is not fully understood in any host species but it is clear that dogs are more sensitive to the compound than are ruminants and the converse is true of their parasites.

A survey of cattle tick control practices in the eastern Cape Province of South Africa

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Current cattle tick control practices and producer attitudes towards tick control in the eastern Cape Province of South Africa are discussed. These were ascertained from answers to a questionnaire survey to which 31,2% of farmers responded. In general, producers favoured intensive tick control. Beef and dairy farmers had a definite preference for synthetic pyrethroid acaricides, the majority followed a 25 times per annum treatment frequency and most changed acaricides because of price. Beef producers favoured pour-on application of acaricides while the majority of dairy producers utilized plunge dipping. Producers who used hand spray techniques experienced the highest percentage of confirmed acaricide resistance. A cost of R11,27 for acaricide treatment per bovine per annum was calculated from data gained in this survey. Only a small number of producers used heartwater, babesiosis and anaplasmosis vaccines. Relative tick borne disease mortality ratios indicated higher heartwater mortalities at high acaricide treatment frequencies.

In vitro establishment and cultivation of a *Cytauxzoon* sp. (*Theileria* sp.) from a sable antelope (*Hippotragus niger*, Harris 1838)

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Examination of blood smears prepared from apparently healthy members of a variety of African antelope species has frequently revealed them to be carriers of *Theileria*-like piroplasms. *Theileria*-like schizonts have only rarely been encountered and their presence has usually been associated with clinical disease and/or mortality of the host, notably in some rare antelope species such as sable and roan antelope. A separate genus, *Cytauxzoon*, was created to accommodate these parasites of antelope taxonomically, but some uncertainty remains with regard to the validity of this genus, and many consider *Cytauxzoon* to be a synonym of *Theileria*.

Upon post-mortem examination of blood and organ impression smears from a sable antelope calf which had apparently died of theileriosis, large numbers of intraerythrocytic piroplasms, as well as schizonts, were observed. Spleen cell cultures were initiated employing standard techniques as used for the in vitro cultivation of *Theileria* spp. Initially, the majority of cells attached to the surface of the culture vessels and became established as monolayers. Following a number of subpassages of these fibroblast-like cells over a period of several months, the number of schizont-infected lymphoblastoid cells growing in suspension gradually increased. Infected cells could subsequently be subcultured at regular intervals whilst maintaining a high infection rate and high cell viability, thus closely resembling transformed *Theileria*-infected lymphoblastoid cell lines.

This appears to be the first successful attempt to establish schizont-infected cell lines from an African antelope species other than African buffalo or eland in vitro, and provides a unique opportunity to study the genetic relatedness of this parasite to the known *Theileria* spp. affecting cattle and possibly develop specific diagnostic techniques.

First record of *Chonopeltis inermis* Thiele, 1900 in the Limpopo River System, with notes on geographical distribution

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With the recent description of 2 southern African species, *Chonopeltis victori* Avenant-Oldewage, 1991 and *Chonopeltis koki* Van As, 1992, the number of species of the endemic African genus *Chonopeltis* now stands at 13. The first, *Chonopeltis inermis* Thiele, 1900 was based on a description of the female only and was followed by a more comprehensive description of the female (Thiele, 1904). The male was later described by Fryer (1956). All the material of *C. inermis* referred to above originated from the mouth cavity of clariid fishes from Lake Malawi.

During surveys of fish parasites in the Luphephe River, a tributary of the Limpopo, specimens of *Chonopeltis* were collected from inside the gill chamber of the snake catfish *Clarias theodorae* Weber, 1897. These showed close resemblance to *C. inermis* from Lake Malawi. To verify this identification, 4 specimens, 2 males and 2 females of *C. inermis* collected by Dr G. Fryer from *Bathyclarias nyasensis* Worthington 1933, in Lake Malawi were obtained on loan from the British Museum (Natural History). This material was compared with *Chonopeltis* collected from Luphephe River by means of scanning electron microscopy. This study showed beyond doubt that the latter is indeed *C. inermis*, implying that this species had dispersed across a watershed to establish successfully in a different system.

Characterization of the 27 kDa and 31 kDa proteins of *Cowdria ruminantium*

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The 27 kDa and 31 kDa proteins are common between 9 stocks of *Cowdria runinantium* which differ in antigenic composition, virulence, pathogenicity, serotype and origin. Thus, these 2 proteins may be key proteins for the development of a suitable diagnostic test or vaccine. These proteins of *C. ruminantium* were characterized and the following observed: Two dimensional electrophoresis indicated that the 31 kDa protein was not contaminated with any other protein of the same molecular weight when crude infected tissue extracts were subjected to SDS-PAGE. Both the 27 kDa and 31 kDa proteins are single polypeptide chains. Isoelectric focusing analysis revealed that the pI of the 27 kDa and the 31 kDa proteins are 5,2 and 5,7 respectively under denaturing conditions. The 31 kDa protein is not a glycoprotein. The amino-acid composition of the 31 kDa protein was determined and indicated a 28 % acidic amino-acid and a 12 % basic amino-acid content. The 31 kDa protein was N-terminally blocked and a partial, internal amino-acid sequence was determined. Monospecific antiserum was prepared against the 27 kDa protein in rabbits and against the 31 kDa protein in rabbits and a goat. Results indicate that the 27 kDa and 31 kDa proteins share common epitopes. The 31 kDa protein did not appear to be protective against heartwater when the immunized goat was challenged with an homologous stock.

The monospecific antiserum and an oligonucleotide, constructed from the partial amino acid sequence, may be used to screen *C. ruminantium* expression and genomic libraries respectively. This may lead to the isolation of the 31 kDa protein and/or corresponding DNA coding sequence for possible use in a diagnostic assay.

Testing antigens of *Rhipicephalus appendiculatus* in calves

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Acquired resistance in laboratory animals, as well as in livestock, to repeated infestation with ixodid ticks is a known phenomenon. It is also known that cattle can be immunized against *Boophilus microplus* by using extracts of this tick as a source of antigens. In Africa, *Rhipicephalus appendiculatus*, the vector of East Coast Fever, is one of the most important ectoparasites of cattle. This 3-host tick does have the capacity to induce resistance in cattle as a result of repeated infestations and immunization may offer many developing countries a potential alternative for its control.

Four of 5 groups of 4 calves each were inoculated with adult mid-gut and nymphal homogenate, nymphal fraction 2 or adjuvant (antigen injected group), while a fifth group was subjected to repeated tick infestation with relatively high numbers of *R. appendiculatus* adults (infestation group) to serve as a control to the inoculated groups.

All 20 calves were finally challenged with the same number of *R. appendiculatus* adults as used in the Group 5 infestation regime. Engorged female tick mass of all groups was subjected to non-parametric Kruskal-Wallis analysis and multiple comparison of mean ranks done with the Dunn method.

Based on the depression of engorged female mass of ticks from the challenge infestation of all the groups, nymphal fraction 2 had a superior immunizing effect than any of the other antigens as well as repeated tick infestation. This effect was, however, not statistically different from that attained by nymphal homogenate and, importantly, a third or a fourth (= challenge) tick infestation.

The seasonal abundance of oribatid mites on an irrigated Kikuyu grass pasture

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The seasonal abundances of oribatid mites, the intermediate hosts of Anoplocephalid cestodes, were studied on an irrigated pasture at the Onderstepoort Veterinary Institute. Twice a month soil and herbage samples were randomly collected 2 hours after sunrise, at midday and 2 hours before sunset and the mites were recovered in an autosegregator.

Although *Galumna nuda, Galumna rasilis, Scheloribates elsi* and *Tectocepheus sarekensis* were the most prevalent mites and were consistently present throughout the study period, each displayed an individual seasonal distribution. On the herbage *G. nuda* showed a peak in October and January, *G. rasilis* in January, *S. elsi* in March, *T. sarekensis* in January while the total numbers of mites peaked in January. On the herbage and in the top 5 cm of soil *G. nuda* showed a peak in December, *G. rasilis* in November, *T. sarekensis* in July and *S. elsi* in April, May and June (1991). Because *S. elsi* was the most abundant species, the total number of mites showed a peak at the same time, i.e. April - June. On the herbage, the total number of mites was significantly higher 2 hours after sunrise than at midday and 2 hours before sunset. There were no significant differences in the total numbers of mites on the herbage and in the top 5 cm of soil at different times of the day.

It is therefore clear that there is a seasonal variation in the abundance of the different species of oribatid mites on irrigated pasture.

The influence of microclimatic factors on the abundance of oribatid mites on an irrigated Kikuyu grass pasture

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The total number of mites on the herbage correlated very well with the temperature in the mat in the morning as well as 30 days prior to collections and with rainfall in the morning and afternoon as well as 30 days prior to collection. On the herbage and in the top 5 cm of soil the total number of oribatid mites correlated negatively with soil temperature in the morning, afternoon and 30 days prior to collection. The numbers also correlated positively with radiation in the morning, at midday, on the day of collection as well as 7, 14 and 30 days prior to collection.

It is therefore clear that environmental factors such as temperature, rainfall and radiation influence the abundance of these mites at different times of the day as well as at different times of the year.

Some short cuts to the post mortem diagnosis of helminths in sheep

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Until recently, helminthiasis was generally not regarded as an important problem in domestic livestock because there were sufficient and very effective anthelmintics available with which to control the common helminths. Also, new anthelmintic groups were discovered with reassuring regularity. Therefore, decisions on the need for drenching were very often taken lightly, without proper diagnosis. The possibility that the helminths concerned could ever develop resistance from excessive drenching was never seriously considered. However, it has become evident that resistance is emerging at a rate that exceeds that of the discovery of new anthelmintic groups. One approach to the problem of resistance is to limit anthelmintic drenches by quantifying infections and thus to ensure that animals are treated only when necessary. Unfortunately, the common methods used for estimating worm burdens are unwieldy, specialized and expensive and therefore seldom used by the practitioner. Trials were conducted to improve the ease with which helminths of sheep can be quantified at necropsy. Preliminary results indicate that > 75 % of *Trichostrongylus colubriformis* occur in the first 5 m of the small intestine. Furthermore, in sheep that are examined shortly after death, > 90 % of *Haemonchus contortus* are found in the small amount of ingesta that adheres to the abomasal wall. Use of this knowledge can provide the practitioner with a considerable short cut for obtaining a reasonably accurate estimate of the extent of helminth infection of sheep at necropsy.

Prospects for improved control of tick-borne diseases in Africa

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The epidemiology of tick-borne diseases is markedly diverse in different areas of Africa. Ticks and tick-borne disease have been investigated over many years in most African countries and they are considered to be of great economic importance to their livestock industries. The economic losses incurred include productivity losses due to tick infestations per se; such losses have been recently studied in Kenya, Zambia and Zimbabwe. As far as losses due to tick-borne diseases themselves are concerned, it is much more difficult to obtain reliable economic data, although some attempts have been made. The conventional tick control measures of dipping and spraying with acaricides that are applied by governments in country-wide programmes are coming under question in several African countries (notably Zimbabwe, Zambia, Kenya, Uganda, Burundi). This is mainly for economic reasons, but in some cases for epidemiological reasons as well. Some African countries are adopting a strategy to encourage the development of endemic stability to tick-borne diseases, which entails a phased reduction in tick control and a good understanding of the epidemiology of the diseases in the areas selected. In indigenous breeds of cattle, in some highly endemic areas, local stock owners have achieved this by accident. The efforts by countries to upgrade their cattle populations by introducing imported genetics have created highly unstable situations in many areas both for tick infestations and tick-borne diseases.

The present generation of vaccines for tick-borne diseases uses living organisms and these have not been widely adopted in many African countries. For example, the infection and treatment immunization method developed for *Theileria parva* is only starting to gain acceptance at present, 20 years after its development. In addition, the blood vaccines for *Babesia*, *Anaplasma* and *Cowdria* that have been available in South Africa and Zimbabwe for many years are only slowly gaining acceptance in other countries. The main problems with live vaccines are cost, the fear of introducing new parasite strains, the lack of an effective cold chain for vaccine delivery and uncommitted and inefficient veterinary services. In addition, the use of private veterinary services is minimal in much of the continent, and the concept of cost recovery from the farming community has not even been proposed in most countries until very recently.

Chemotherapy has been developed for most of the tick-borne diseases, but it is generally very costly and its efficient application requires accurate and rapid diagnosis.

Africa requires in the years into 21st century to develop robust integrated control of ticks and tick-borne diseases which would include:

1. New and rational methods of tick control which could include strategic acaricide application, genetically-engineered tick vaccines and the use of tick and tick-borne disease resistant cattle.
2. More efficient non-living vaccines for all the important tick-borne diseases which ideally do not require a cold chain. This will involve the development of genetically engineered antigen vaccines. In the case of *T. parva*, this could require antisporozoite, antischizont and transmission blocking components. The appropriate choice of adjuvants and vaccine vectors will have to be considered.
3. New approaches for the delivery of animal disease control measures which are economically viable and sustainable need to be developed. Under this heading, it is required to undertake epidemiological studies at site, country and regional levels, using new technologies; more specific and robust serological tests and nucleic acid probe techniques, carried out with properly structured sampling of livestock populations and methods of determining the accurate tick vector/parasite/host population dynamics. This will permit an accurate assessment of the impact of new control interventions and such studies will support the development of useful and accurate epidemiological and economic models to evaluate control options where only minimal data is available.

Cowdria ruminantium infection in bont ticks, *Amblyomma hebraeum*: detection by means of a specific DNA probe, pCS20

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A DNA probe, pCS20, previously described for use in detection of *Cowdria ruminantium* infections in *Amblyomma variegatum* (the principal vector of heartwater) hybridized with *C. ruminantium* DNA in organs of laboratory-infected *A. hebraeum* adults (the major southern African vector). Ticks had been exposed to the organism as nymphs 5½ months previously through feeding on a febrile sheep infected with Crystal Springs heartwater stock. Infection in ticks was confirmed by transmission of the disease to susceptible goats. The probe hybridized with *C. ruminantium* DNA in 46/49 midguts dissected from male ticks and in 26/29 midguts from females. Corresponding salivary glands were less heavily infected, but infections were more numerous in glands from males. Heat-shock (incubation of ticks at 37°C for 3 days) did not increase the percentage of infected salivary glands from females, nor did it significantly increase the percentage of infection in midguts of either sex. However, the percentage of infected salivary glands in