Parasitological Society of Southern Africa

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Abstracts of Plenary Papers

The role of cytokines in human infectious diseases

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The pro-inflammatory cytokines IL-12, IFN-g, and TNF-a, amongst others, play an important and decisive role in host protection against infectious diseases. This process starts immediately after infection occurs. Some of these cytokines are also of crucial importance to activation of potent effector 'killing' functions, such as reactive nitrogen and oxidative intermediates. Moreover, anti-inflammatory cytokines, like IL-4, IL-10, and IL-13 are not only counter-players to pro-inflammatory cytokines but they also contribute in regulatory processes that direct host responses to gain long lasting immunity. Using mice deficient for cytokines or their receptors, we studied cytokine functions in experimental models of the infectious diseases, tuberculosis, leishmaniasis, schistosomiasis, trypanosomiasis and helminth infections. The major highlights of this research are summarised, including the role of those cytokines in inflammation, T helper dichotomy and activation, chronic disease and expulsion.

Ascariasis: medical and public health significance

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Ascaris lumbricoides has recently assumed a new medical and public health significance, information having become available which shows the need to view human *Ascaris* infection in a new light. Topics to be discussed include the following: medical complications of ascariasis fecundity of *A. lumbricoides* and its implications for helminth control and cerebral malaria; the immune response to *Ascaris* infestation and its importance in relation to HIV/AIDS, tuberculosis and vaccination against various diseases (Markus and Fincham, 2001, *Science* 291: 46; Markus and Fincham, 2001, *Lancet* 357: 1799) immunoregulation in chronic ascariasis and reduced prevalence of atopic disease immunogenetics and disease susceptibility. Eradication of *A. lumbricoides* is not feasible in many countries where ascariasis is endemic. Therefore, the emphasis has to be on control.

Effect of schistosomiasis and HIV co-infections on immune responses in humans

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In vitro studies suggest that CD4+ cells with a Th2 phenotype support HIV-1 replication better than do Th1 cells. As a result, Th2-type immune responses may be substantially affected by HIV-1 co-infection. To test this hypothesis, we compared proliferation and cytokine production by peripheral blood mononuclear cells from schistosomiasis patients who were positive or negative for HIV-1. Schistosomiasis patients with HIV-1 co-infections had significantly lower IL-4 and IL-10 production than did HIV-1 negative individuals. In contrast, IFN-γ production levels were similar between the 2 groups. Furthermore, in patients with HIV-1, a decrease in CD4+ T cells was correlated with an increased Th1/Th2 cytokine production ratio. Response patterns before and after Praziquantel treatment of *Schistosoma mansoni* infection also differed between HIV-1 infection groups. Thus, HIV-1 infection affects immune response patterns of schistosomiasis patients. This may have implications for resistance to infection/re-infection with schistosomiasis in these individuals. We also investigated the intensity and nature of immune responses to parasite antigens as well as the regulation of those responses in relation to the development of fibrotic pathology. We investigated the differences in the immune response profiles of persons with no liver fibrosis compared with persons that have schistosomiasis-related pathology, and how this may be affected by a patient's HIV status. The proportion of patients with fibrosis as detected by ultrasonography was lower in HIV seropositive than in HIV seronegative groups. Mean CD4 levels were significantly lower in persons with severe liver pathology than in the less affected groups after controlling for HIV status but CD8 counts were similar in patients with or without fibrosis. IL-10 and IL-4 but not IFN-g production in response to Schistosome-derived egg (SEA) and adult worm (SWAP) antigens were significantly decreased in the severe pathology group, implying that schistosome specific responses are down regulated in persons with hepatic fibrosis. The presence of HIV down regulated these responses further. However, chemotherapeutic treatment of hepatosplenic patients did elevate IL-4 and IL-10 production in response to both schistosome-derived antigens.

Development of partnership programmes for mass-deworming

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Over two billion people in the world are infected with helminths. Hookworm, whipworm and the large intestinal roundworm are the 3 main intestinal worms. Scientific evidence shows that treating moderate to heavy helminth infections can improve health, growth, and reduce the transmission of infections. Systems currently available can deliver simple, inexpensive and effective treatments to communities most at risk of these infections. Mass-deworming (in different forms) has been identified as the effective and cost-efficient control method. However drugs are only 1 element in parasite control programmes. The necessity of development of partnerships for parasite control programmes is well recognised. The purpose of such partnership programmes is to bring together as many elements as possible (such as education, training, nutrition, clean water supply), which are required to make the efforts sustainable and more efficient. Empowerment of local communities and effective communication and mobilisation strategies deserve a very high priority. Local communities must feel responsible for their own destinies. Other members of the partnerships can be most effective by providing support based on the demands of informed and mobilised communities.

Scale and inferences in parasite molecular phylogenetics and co-evolution

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The combined use of morphological and molecular data sets in the study of the phylogenetic relationships of parasitic groups has revolutionised our understanding of the sister-group relationships of, and thus the origins of, many parasitic lineages (*e.g.*, Myxozoa, Acanthocephala and Haplosporida). Beyond these straightforward issues of evolutionary relationships, parasite phylogenies are useful for examining historical patterns that may be evident in parasite ecology, specialisation, the evolution of life-history strategies, as well as (when host phylogenies are available) host-parasite co-speciation and host-switching. Several examples are presented, ranging from the phylogeny of viruses (HIV), to protozoa (malaria

parasites and flagellates) to metazoans (Nematoda and leeches) and are discussed in terms of the need to be circumspect about the scalability of early findings in the face of increasing taxonomic scope and the addition of character information.

African human trypanosomiasis (sleeping sickness): overview and control

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Human African trypanosomiasis (HAT), which is also known as sleeping sickness (SS), is caused by protozoal haemoflagellates *Trypanosoma brucei gambiense* and *T. b. rhodesiense* and is transmitted by tsetse fly (*Glossina* spp). SS is an epidemic-prone infectious disease confined to sub-Saharan Africa. It currently kills over 100 people daily. SS was virtually eliminated from African core countries in the 1960s. It started to return in the 1970s and 1980s, with major resurgence in the 1990s. SS has become a major threat to public health. SS epidemics currently exist at the Rift Valley border between regions that are endemic for *T. b. gambiense* and *T. b. rhodesiense.* These span 4 war-torn countries. Current epidemics are mainly Gambian SS. Cases have also been reported in countries formerly at war or stricken by catastrophes, and in refugees from epidemic areas. The exact situation is not clear because reports are irregular and unreliable. Overwhelming reluctance to acknowledge SS and act in time is hindering control in communities where malaria, meningitis and HIV/AIDS are the first-line killers. Early achievements of habitat modification and insecticide-based tsetse and trypanosomiasis (T&T) control were not maintained. Inability to prevent re-invasion, maintain surveillance, resistance to drugs, poor access to effective drugs and lack of cooperation between high-risk countries, nurtured active foci. Subsequent control efforts not only failed for environmental reasons, but also lacked regional cooperation and community engagement. A long-term solution in epidemic-stricken countries is elusive. While national control programmes operate within 'safe' foci, only international non-governmental organisations dare to treat or watch those within 'dangerous' foci. The conception of the Programme Against African Trypanosomiasis and its engagement of commitment from political leaders in the Organisation for African Unity, especially to regional cooperation in T&T control, was a good start. Where war does not prevail, collaboration was established, high-level scientific research and technology applied, resident communities motivated and rural development projects initiated. The European Union-funded Regional Tsetse and Trypanosomiasis Control Programme could offer a long-term solution with emphasis on high-level political interaction between Uganda, the Sudan, the Democratic Republic of Congo and Angola. Multitudes of poor people in climate-unfriendly, war-torn Africa are dependent on foreign aid and are dying from SS. T&T control achievements elsewhere in Africa could be jeopardised by the uncontrolled epidemics.

Origin and phylogeny of leeches, with notes on the evolution of anticoagulants

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Leeches are annelids with an ectoparasitic lifestyle in most cases. The phylogenetic relationships of major families of leeches were investigated by means of combinations of morphological and molecular data sets. Most of the useful morphological characters were obtained from sexual and coelomic anatomy. On their own, however, these features were of limited value. Preliminary analyses that combined these data with 18S rDNA and CO-1 nucleotides succeeded in supporting most groups as monophyletic. With the subsequent addition of various South American transitional leech taxa, fully supported phylogenetics required the addition of 28S rDNA and of 12S rDNA. Results indicate that leeches are divisible into the families Glossophonidae, Ozobranchidae, Piscicolidae, Americobdellidae, Erpobdellidae, Macrobdellidae, Haemadipsidae, Xerobdellidae, Hirudinidae and Haemopidae. Moreover, the phylogeny indicates a minimum of 5 losses of blood-feeding in the evolutionary history of leeches. Use of the 18S rDNA and CO-1 data from leeches together with comparable information from more than 130 annelids, demonstrates that leeches, branchiobdellidans (crayfish worms) and *Acancthobdella* arose from a common lumbriculid oligochaete ancestor. That is, leeches *are* oligochaetes. Careful consideration of the distribution of anticoagulants in a phylogenetic context, suggests that the early mechanism of preventing blood from clotting involved a platelet aggregation inhibitor. Potent thrombin inhibitors appear to have originated later in leeches ancestral to the Hirudinidae, Haemadipsidae and Haemopidae.

Oral Presentations

Developments in helminth systematics

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According to Longman's Dictionary of Contemporary English, an odyssey is a long journey with lots of adventures. The Oxford Dictionary describes an odyssey as a series of wanderings, or a long adventurous journey. This presentation is about the long journey in the developments of systematics of especially the nematode parasites of animals. Initially, when worms were still seen as strange and evil creatures, the names given to and the illustrations of the species were rather imaginative. Another generation of taxonomists made very detailed drawings and provided measurements, both of which enabled the worms to be identified with relative ease. This was (and to a large extent still is) the only method to separate and identify worm species. Describing new species became quite popular during the seventies and eighties, but not all the information pertaining to these species was provided and illustrations were of a poor standard. The difference in morphological features when the worms are not exactly orientated in the same position lead to confusion and the International Commission for Zoological Nomenclature had to impose certain rules. As the technology developed, new techniques were devised by which helminths could be identified more accurately, for instance, the transverse synlophe, the longitudinal synlophe and the arrangement of the circum-oral papillae. The former 2 are applicable only to the Trichostrongyloidea, while the last-named are applicable to all nematodes. These techniques were further refined and together with electron microscopy showed that the variation between nematodes even within a genus is quite apparent. The new approach to systematic helminthology is by means of molecular techniques. These techniques have been used with great success in other disciplines and have comparatively recently been applied to helminths. Techniques include the polymerase chain reaction (PCR) and DNA sequencing. Some examples are provided to illustrate the differences in the arrangement of nucleotides whereby species can be accurately identified. For some the odyssey is ending, but for others it just beginning, notably in the field of molecular helminthology. The latter is a powerful tool in our understanding of the evolution and phylogeny of species and could identify aspects of problem species such as *Haemonchus contortus.*

Helminth control practices on Thoroughbred horse farms in South Africa

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A questionnaire was developed to obtain information on the helminth control practices and management on Thoroughbred horse farms in South Africa. It was sent to 110 stud farms during 2000. The farms consisted of a minimum of 15 animals and were distributed all over South Africa. A total of 57 questionnaires (52 %) were returned and most of these (76 %) came from farms located in 2 provinces (Western Cape and KwaZulu-Natal). The average number of horses per farm was 105 (±80.03) with a minimum of 15 and a maximum of 410. The study revealed that foals, yearlings and

adults were treated 7.34 (±2.97), 6.61 (±2.69), and 5.27 (±2.31) times per year, respectively. An average of 3.36 different drugs was used annually with ivermectin being the most commonly used for the period 1997–2000. To obtain the body weight estimates, in order to gauge the dose of anthelmintic, the eye measure was used on 45 % of the herds and the body tape measure on 43 %. Individual weights and the average group weight were equally preferred at dosing. Most of the herds (81 %) were subjected to faecal egg counts 3.32 times per year. Ninety percent of herd owners practised pasture rotation, 43 % performed alternated grazing with cattle, 35 % spread the horse dung on the pastures while 61 % removed dung from the pastures. Thirty-four percent performed anthelmintic treatment at rotation, 95 % treated new horses at introduction and 78 % treated visiting horses. Colic seems to be of little importance following deworming and 82 % of owners performed disease registration.

Parasitic helminths of veterinary importance in the northeastern Free State, South Africa

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Veterinary helminths are parasitic worms infecting livestock, mostly entering their hosts through ingestion and escaping in the faeces as eggs. The purpose of the study was to record helminth parasites that occur in cattle, sheep and goats in the northeastern Free State. The study was conducted in 3 sites, Harrismith, Kestell and Qwa-Qwa, for a period of 12 months from March 2000 to April 2001. Faecal samples from 531 cattle of various breeds that included Bonsmara, Simmentaler and Friesian, 449 Merino sheep and 242 Angora goats were analysed using the McMaster and Visser sieve techniques for identification of the eggs and counts of eggs per gram (epg), and by faecal cultures for L_3 identification. Dominant species infesting cattle were found to be *Dicrocoelium lanceatum* (epg range: 50–4800) and *Paramphistomum cervi* (epg range: 33–1200) while in small stock dominant species were found to be *Haemonchus contortus* (epg range: 100–2800) and *Ostertagia circumcincta* (epg range: 100–5550). Parasitic infections followed a seasonal pattern, with high epgs recorded during the warmer months of the year (November–February), while in the colder months of the year (May–July) lower epg's were recorded. Results of a questionnaire survey conducted during the study period indicated that farmers do not regard helminthiosis as a major problem.

A new cestode from the intestine of *Clarias gariepinus* (Burchell, 1822)

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This paper gives a brief description of a previously unknown cestode from the intestine of *C. gariepinus*. Specimens of *C. gariepinus* were collected from Loskop Dam using gill nets. Fish were transported alive to the field laboratory where they were killed immediately prior to being examined for parasites. Cestodes were killed and fixed in alcohol-formal-acetate (AFA) and preserved in 70 % alcohol. Whole mounts were stained in Alum Carmine and sections in Azan. They were dehydrated in a series of alcohols, cleared in xylene and mounted in Entellen. A very large cestode with the following description was collected from the intestine of fish species. Scolex with 4 acetabular suckers; rostellum and metascolex absent. Vitellaria follicular, lateral and medullary; testes medullary; ovary and uterus partly medullary and partly cortical; genital pores alternate irregularly and open on the lateral margin of proglottid in the anterior third; cirrus sac transversely situated, opening separately from either anterior or posterior to vaginal opening; cirrus unarmed, long; testis round, ±270 in each proglottid, anterior to ovary; latter bilobed, in posterior third of proglottid, parallel to posterior margin of latter. Uterus median with 6–10 lateral branches on each side. The cortex and the medulla is separated by a very thick and well-developed muscle layer. This parasite has many similar characteristics with members of the subfamily Proteocephalinae, but differs from them in that the ovary and uterus are partly cortical and partly medullary. In this respect, it resembles members of the family Monticelliidae but differs from the latter in that the vitellaria are medullary and not cortical as in the family Monticelliidae.

Polystomatid marginal hooklet as taxonomic character

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Among the fifty monogenean families the Polystomatidae is the only one that radiated onto tetrapods and is today mainly parasitic in amphibians. Polystomatids are known for little interspecies and large intraspecies variation. As with most other monogeneans, the size and shape of sclerotised structures of polystomatids are crucial in the identification of species. The majority of the polystomatid genera have large hamuli, however the size and shape of these structures are also known to vary within some species. A more reliable character appears to be the marginal hooklets that are retained in the adult form and appear to be fairly constant in shape. The posterior-most pair of the 16 marginal hooks, referred to as hooklets (C1) is the largest and are traditionally measured. As part of this study 13 measurements of 10 marginal hooklets from 13 different *Polystoma* species were studied. Hooklets were digitally photographed and measured using the *Scion* image analysis computer programme. The aim of this study was to study the marginal hooklets and evaluate different techniques and measurements as taxonomic characters and to propose a protocol for future species descriptions. This study revealed that the marginal hooklet is very valuable as a taxonomic character. Limited intraspecies morphological variation exists while interspecies differences are of such a nature that comparing sets of measurements could help significantly to separate species.

Pentastomid parasites from Nile crocodiles and terrapins from South Africa

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Pentastomes are endoparasites maturing in the respiratory tract of their final hosts, more than 90 % of which are reptilians. Of the existing 8 families of pentastomes, 2 families, the Sebekidae and Subtriquetridae infect crocodilians, using fish as intermediate hosts. Currently, the family Sebekidae comprises the 6 genera, *Sebekia* Sambon, 1922, *Leiperia* Sambon, 1922, *Alofia* Giglioli, 1922, *Selfia* Riley, 1994, *Agema* Riley, Hill & Huchzermeyer, 1997 and *Diesingia* Sambon, 1922. The latter genus is an exception insofar as it has a chelonian definitive host. Seventeen Nile crocodiles (*Crocodylus niloticus*), were obtained from the Kruger National Park in 1995, 1997 and 1998. Sixteen of these (1.4 to 4.15 m long) harboured pentastomes. No difference in the pentastome fauna was found between the sexes and between ages (as determined by length). All pentastomid parasites recovered belonged to the family Sebekidae. Three *Sebekia* spp. were collected, namely *S. cesarisi, S. okavangoensis* and *S. wedli*. Of the genus *Alofia, A. nilotici* and *A. simpsoni* were present. The genus *Leiperia* was represented only by *L. cincinnalis*. Subtriquetrids were not found. The species composition at different localities appeared to be fairly homogenous, with a slight dominance of *S. wedli* and *L. cincinnalis* over the other species. *Alofia* was the least numerous and least prevalent of the 3 genera. All but 1 crocodile carried multiple infections. The intensity of infection varied from 2 to 239 pentastomes per host, with an average of 40. With the exception of 1 moribund crocodile, whose condition was attributed to an extremely heavy infection with pentastomes, Nile crocodiles seem to tolerate pentastome infections well. Terrapins, *Pelomedusa subrufa* and *Pelusios siniatus* were obtained from Arabie Dam, Limpopo Province. Examination of the pentastomes recovered from the lungs of these animals led to the description of a new species, *Diesingia africana*, which is considered endemic to African terrapins. The genus *Diesingia* Sambon, 1922, was reconfirmed and is reported for the first time in Africa. Our field studies indicate that pentastomes are common parasites of crocodiles in the Kruger National Park. The

host–parasite relationship is well-established, suggesting a long association between the parasite and its host. However, exceptional environmental conditions can lead to abnormally high pentastome burdens, which severely affect the host's condition. Despite recent studies, much is still to be learnt about pentastomes infecting aquatic reptilians in South Africa, especially terrapins.

A protective and agonistic function of interleukin-12p40 in mycobacterial infection

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Recent findings by us, and others, indicate a protective and agonistic role of IL-12p40 in some infectious disease models. Using gene-deficient mice as an animal model we investigated the role of IL-12p40 in mycobacterial infections. Interleukin (IL)-12, the key factor for the release of gamma interferon (IFN-γ) by natural killer (NK) and Th1 cells, is known to be produced by antigen presenting cells upon interaction with mycobacteria. It has also been shown that IL-12 has direct stimulating effects on effector functions of $CD8^+$ T cells which are critical for an effective control of mycobacterial infection. Bioactive IL-12 is a 70-kDa heterodimer consisting of a 35-kDa (p35) and 40-kDa (p40) sub-units. IL-12p40 is known for its antagonistic properties due to its competing activity with the IL-12 receptor. However, recent studies by us, and others, revealed the possibility that IL-12p40 does have some agonistic properties in the absence of bioactive IL-12. To evaluate the role of endogenously produced IL-12p40 in mycobacterial infections, comparative infectious studies with *Mycobacterium bovis* Bacillus Calmette-Guerin (BCG) or *Mycobacterium tuberculosis* infected IL-12p35-deficient mice ($IL\text{-}12p35^{-/-}$), or IL-12p35/p40 double-deficient ($12p35^{-/-}/p40^{-/-}$) mice and wild-type controls were performed. Mutant and wild-type mice were infected with 2×10^6 colony forming units (CFU) BCG intravenously or with the virulent *M. tuberculosis strain* (H37Rv) in an inhalation exposure system with 10^7 bacteria, resulting in 100 CFU/lung. Kinetic analysis of bacterial loads and histopathology of infected organs, determination of antigen specific T cell functions using delayed type hypersensitivity (DTH), proliferation assays, cytokine responses during *in vitro* restimulation, T cell cytotoxicity assays and *in vivo* therapy with recombinant IL-12p40 were performed. $IL\text{-}12p35^{-/-}$ mice, which are able to produce endogenous IL-12p40, cleared *M. bovis* and showed evidence for resistance to pulmonary *M. tuberculosis* infection. In striking contrast, $12p35^{-/-}p40^{-/-}$ mice were highly susceptible to *M. bovis* BCG and *M. tuberculosis* infection. Resistance in wild-type and $IL\text{-}12p35^{-/-}$ mice was accompanied by protective granuloma formation and an antigen specific DTH response, which were both impaired in $IL\text{-}12p35^{-/-}/p40^{-/-}$ mice. Furthermore $IL\text{-}12p35^{-/-}$ but not $IL\text{-}12p35^{-/-}/p40^{-/-}$ mice were able to mount antigen-specific Th1 and cytotoxic T cell responses. *In vivo* therapy with IL-12p40 homodimer restored the impaired DTH responses in *M. bovis*-infected $IL\text{-}12p35^{-/-}/p40^{-/-}$ mice and reverted them to a more resistant phenotype. Together these results demonstrate a protective and agonistic role of endogenous and exogenous IL-12p40 in mycobacterial infection that is independent of IL-12p70. Currently, we are investigating whether $IL\text{-}12\ (p40)_2$ may be used as an immune adjuvant to optimise BCG vaccination.

Latency and reactivation of tuberculosis: a murine model

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Mycobacterium tuberculosis (Mtb) causes active tuberculosis in approximately 30 % of infected persons. In the majority of cases an effective immune response allows containment of Mtb, also known as latency (estimated to affect one-third of the world population). Reactivation is caused by immunosuppression in 10 % of these individuals. Very little is known about the host–bacillus interaction during latency and reactivation, and experimental studies lack appropriate animal models. A murine model of latency and reactivation had to be established, mimicking human latency and reactivation. C57BL/6 wild-type mice and Tumour Necrosis Factor gene-deficient mice were aerogenically infected with 30 colony-forming units (CFU) *Mtb* H37Rv per lung. Infection was allowed to establish for 2 weeks, after which mice were treated with 0.1 g/ℓ rifampicin (RMP) and isoniazid (INH) for 4–12 weeks to achieve latency. Reactivation of infection was obtained with 2.5 % aminoguanidine (AG), an inhibitor of inducible nitric oxide synthase (iNOS). Lung, liver and spleen samples were harvested from mice at various time points and the bacterial load and histopathology assessed. *Mtb*-infected mice showed an increase of 2.5 logs CFU in lung tissue 2 weeks post-infection. Latency was achieved 4–12 weeks post-RMP-INH chemotherapy. Mice showed increasing body weights, remained healthy and had no culturable CFU in lung, liver or spleen. Reactivation could be induced in 50 % wild-type mice following AG administration after 8 weeks antituberculous chemotherapy and latency for 20 weeks. Both gene-deficient (100 %) and wild-type (100 %) mice spontaneously reactivated following 4 weeks of chemotherapy. Following spontaneous reactivation, gene-deficient mice died within 7–11 weeks (0 % survival), although wild-type mice contained the infection (100 % survival). Histological findings showed granuloma formation in both knockout and wild-type mice, although knockout mice have smaller granulomas and delayed formation at comparative time points. A mouse model for latency and reactivation of tuberculosis has been established and will form the basis for elucidation of the host immune response, including macrophage recruitment, cytokine profiles and distribution of T-cell populations. In addition, this model can be used in pre-clinical testing of novel anti-tuberculous drugs and vaccines.

Innate and adaptive immunity against *Schistosoma mansoni* downregulates protective interferon gamma (IFN-γ) responses against *Listeria monocytogenes*

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Innate and adaptive immunity against microbial pathogens as well as parasitic helminths involves both the recruitment of distinct populations of effector cells and restricted cytokine production profile. This initial cytokine milieu generated by the host is critical for the development of long-lasting adaptive immunity mediated by T cells. $CD4^+$ T cells serve an integral role in acquired immunity against many different types of pathogens and can be divided into 2 functionally distinct repertoires. T helper 1 (Th1) cells are characterised by the production of IFN-γ and are associated with the presence of natural killer cells, macrophages, and neutrophils, all of which serve a function in protection against most microbial and viral pathogens. In direct opposition, are the Th 2 cells, which produce IL-4, IL-5 and IL-13 that are associated with the presence of mast cells, eosinophils, and basophils and protection against parasitic helminths. Th1 versus Th2 responses can be observed following infection with the *Human Immunodeficiency Virus* (HIV) or the parasitic helminth *Schistosoma mansoni*, respectively, both of which cause significant morbidity and mortality in human populations worldwide. Although there are many regions in Africa, Asia and South America where people may harbour both pathogens simultaneously, it is not known whether counter-regulation of protective responses occurs in such doubly infected persons. In the present study, we addressed the hypothesis that cells of the innate immune response could counter-regulate each other during a response to double-infection with Th1 and Th2 pathogens. The model organisms used in this study were the intracellular bacterium *Listeria monocytogenes* and the trematode parasite *S. mansoni*. Wild-type BALB/C or C57BL/6 mice were injected intraperitoneally with either 500 cercariae or eggs of *S. mansoni* 24 hours before infection with 10^4 colony forming units (CFU) of *L. monocytogenes*. Peritoneal exudates cells (PEC) were isolated at day two or day-seven post-*Listeria* challenge to analyse innate and adaptive immunity, respectively. PEC were identified by differential cell staining and analysed by FACS to determine their potential to release Th1 or Th2 cytokines. In addi-

tion, the spleen and liver of infected mice was removed to determine the number of CFU at each time point examined. Results indicate that the innate response to *S.* mansoni eggs or cercariae involves the recruitment of predominantly macrophages and mast cells within 24 hours of initial exposure. Furthermore, we found GR-1 (Ly-6G) positive cells that could release IL-4 at both day 2 and day 7 post-infection. By contrast, *Listeria* infected wild-type mice showed a recruitment of neutrophils and cells that were positive for intracellular IFN-γ. This correlated with a virtual clearance of bacteria from the spleen and liver by day 7 post-infection. However, mice that received both *Schistosoma* and *Listeria* produced PEC that were IL-4 positive and few that were IFN-γ positive. Also, there were significantly higher bacterial counts in the spleen and liver of doubly infected mice in comparison to those infected with only *Listeria* at day 2 and day 7 post-infection. In conclusion, the protective immune response to an intracellular bacterium *L. monocytogenes*, which involves the recruitment of neutrophils and the production of IFN-γ against *L. monocytogenes* is downregulated by the presence of cercarial or egg antigens from the parasitic helminth *S. mansoni*. This study indicates that Th1/Th2 cross-regulation could occur at the level of innate immunity and is not only limited to the T-cell response.

The functional micromorphology of the goat-biting louse (*Bovicola caprae*)

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Bovicola caprae is a common ectoparasite of goats worldwide. The biting mouthparts are used to scrape epidermal debris causing severe irritation and the symptomatic scratching by the host. The micromorphological specialisations of *B. caprae* were investigated by scanning electron microscopy (SEM). Live lice collected from indigenous goats in the Limpopo Province were fixed directly in 70 % ethanol. After ultrasonication, specimens were routinely processed and viewed in a Leica Stereoscan 420 SEM at 5 to 7 kV. The SEM study revealed several specialisations not visible on slide-mounted specimens. The mandibles by which the lice attach to the hairs of the host frequently had double-notched cutting edges. The lip-like epipharynx is covered by many dentate scales, which suggests a rasping or scraping function. The 3 segments of the antennae of the adults are similar in structure but sexual dimorphism was seen in that the antennae of the male has many more setae giving it a hairy appearance. In addition the distal segment of the male has 2 robust conical setae medially near the tip, that function for grasping the female during copulation. The peg organ at the distal tip of the antenna consists of 12 sensilla of varying lengths. Adjacent to the 2 pore organs that each contains a tuft organ, are 3 small plate organs. The sclerotised thorax consists of a fused pro- and mesothorax and a separate metathorax. The luminal surface of the large thoracic spiracles located laterally on the mesothorax, is lined with honeycomb-shaped lamellae. Each leg terminates in a single slender claw that closes against an appositional thumb-like, disto-tibial setae which forms a 'grasping organ' for attachment to the host's hair. A single layer of short setae extends across the posterior edge of each abdominal segment I to VII. Fine-toothed projections were observed on segment VIII as well as the sternites of segments II to VI. The male and female terminalia were confirmed to be strongly sexually dimorphic. Two flaps close the genital opening of the male dorsally. The flaps are covered by many short, hooked setae so as to resemble pincushions. The female has a pair of characteristic posterolateral flaps that may help in placing the egg on the hair during ovipositing.

Mycobacterium tuberculosis infection in lymphotoxin-deficient mice

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Mice that express tumour necrosis factor alpha (TNF) and others that are deficient in this factor (TNFR-1) are both susceptible to infection by *M. tuberculosis*. Since TNF and lymphotoxin alpha (L Ta) both signal through TNFR-1, the role of L Ta in host resistance is not resolved. In order to address this question, we investigated the progression of infection after aerosol H37Rv mycobacterial strain infection, with about 100 colony forming units (cfu)/lung, in L Ta-deficient and irradiated wild-type (WT) mice reconstituted with bone marrow cells from L Ta-deficient or wild-type mice. We demonstrated that L Ta-deficient mice are as susceptible to mycobacterial infection as TNF-deficient mice and succumb within 5 weeks after H37Rv mycobacterial infection. Irradiated WT mice reconstituted with WT bone marrow, survived for the duration of the experiment. Determination of mycobacterial loads in lung indicated significantly higher cfu levels in L Ta-deficient compared to Wt mice. The lung pathology in L Ta-deficient mice, revealed a progressive pneumonia with severe necrosis. The number of cfu in spleen and liver, and histology, indicated haematogenous spread of mycobacteria and thus an inability to contain infection in L Ta-deficient mice. Furthermore, the formation of granulomas was clearly delayed in L Ta-deficient mice. In conclusion, L Ta is required and cannot be compensated by TNF to mount a protective immune response against H37Rv mycobacterial infection.

A preliminary study on acaricide resistance profiles of single and multi-host ticks collected from commercial and communal farming areas in the Eastern Cape and North West provinces of South Africa

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A field study was carried out from January to March 2001, on selected communal and commercial farms in the Eastern Cape and North West provinces of South Africa, to detect the levels of tick resistance. The larvae obtained from engorged females of *Amblyomma hebraeum, Boophilus decoloratus, Rhipicephalus appendiculatus,* and *Rhipicephalus evertsi evertsi* were tested by an established method against different concentrations of amitraz 12.5 %, chlorfenvinphos 30 % and cypermethrin 15 %. Mortality dose data were subjected to probit analysis using the BMDP statistical package. Factors of resistance were calculated based on the larval response of tick species from the field, which had been exposed to an acaricide and were compared with baseline data obtained from the most susceptible strains on the basis of the LC_{50} value. Preliminary results indicated that ticks from commercial farms were considerably more resistant to amitraz than cypermethrin and chlorfenvinphos. However, on the communal farms, higher levels of resistance was shown to cypermethrin and some resistance to chlorfenvinphos while no resistance was detected against amitraz. The population of *B. decoloratus* was considerably more resistant to all acaricides tested than the multi-host ticks *A. hebraeum, R. appendiculatus* and *R. evertsi evertsi*.

A comparative SEM study of Haller's organ in 3 African *Amblyomma* tick species

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Theiler (1962) recognised 21 *Amblyomma* species in the Afrotropical Region. Although the adults of most of the *Amblyomma* species parasitise livestock and/or large mammals, some prefer the reptile host. The survival of a tick depends on how successfully it can locate a prospective host. Host location is facilitated through Haller's organ situated on the dorsal surface of the tarsi of appendage I. This unique organ consists of sensory setae and sensilla, which are strongly innervated. Besides olfaction, some of these sensilla have

been found to function in a hygrosensory, mechanosensory, thermosensory and gustatory role. Adults of *A. hebraeum* and *A. gemma* that parasitise livestock and large ungulates, while those of *A. marmoreum* are found on tortoises and large varanids (monitor lizards). Males and females of each species were preserved in 70 % ethanol. The 1st appendages were removed and prepared for viewing in a Leica Stereoscan 420 scanning electron microscope (SEM) at 5 to 10 kV. The composition and ultrastructure of Haller's organ as it is expressed in the 3 species are compared. In spite of the differences in host prevalence no differences in the number and grouping of the sensilla could be found. Obvious differences in the microstructure could, however, be demonstrated.

Parasites of veterinary importance in small stock in the northeastern region of the Free State province, South Africa

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A sero-epidemiological survey was conducted between September 1999 to August 2000 in the northeastern region of the Free State province of South Africa to determine parasites of veterinary importance infesting sheep and goats. Blood smears from sheep (n = 371) and goats (n = 188) were negative for *Anaplasma* and *Theileria*. All sheep and goats were seropositive for *Theileria* species by IFAT. All the goats and 80 % of the sheep were seropositive for *Anaplasma* species by competition inhibition ELISA. The observation of the negative blood smears but high incidence of positive serological results for *Anaplasma* species and *Theileria* species for the 2 animal groups indicates that this area is endemic but with a stable disease condition for these 2 diseases. Two tick species found to infect sheep and goats in the 3 study sites of Harrismith, Kestel and Qwa-Qwa were *Rhipicephalus evertsi evertsi* and *Boophilus decoloratus*. These 2 are known vectors of *Anaplasma* species and *Theileria* species, the 2 diseases found to infest sheep and goats in this region. *Rhipicephalus evertsi evertsi* was the dominant tick species across the study sites. A seasonal pattern in tick infestation was observed with a high peak during the warmer months and a low peak during the colder months.

The functional micromorphology of the spiracles of the adult tortoise tick *Amblyomma marmoreum*

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Spiracles in ticks control both respiratory gas exchange and tracheal water loss. The large size of the spiracles of *A. marmoreum* make them suitable for morphological studies to investigate how the spiracles may control these processes. Fifty per cent of the adult ticks were fixed directly in 70 % ethanol while the remaining 50 % of the flat and engorged ticks were euthanased in CO_2 in an attempt to cause the spiracles to open before fixation. Spiracles were carefully dissected and routinely processed for scanning electron microscopy and viewed in a Leica Stereoscan 420 SEM at 5 to 7 kV. The external morphology revealed a spiracular plate with a porous comma-shaped outer margin perforated by many small aeropyles, surrounding a macula with a central ostium plugged by a valve-like lip. The interpedicellar space consists of a labyrinth of pedicels that connect the spiracular plate to the thick base plate that forms the floor of the subostial space. The pedicels are arranged in groups forming tubular chambers many of which open to the atmosphere through the aeropyles. All chambers interconnect by means of slit-like fenestrations between the pedicles to allow respiration. The subostial space leads into the atrial chamber from which the main tracheal trunks arise. The thick lateral wall of the atrial chamber has a distinctive circular internal rim against which the thin medial wall may evert, to form a valve-like mechanism. This may close off the atrial chamber and trachea from the subostial chamber, and thus limit tracheal water loss through the open aeropyles when the spiracles are closed during inter-respiratory intervals. The dense arrangement of pedicles and the closely-spaced, small aeropyles may further increase the resistance to transpiration and reduce the airflow, thereby reducing the water vapour loss from the spiracles.

Preparation of fluid and mucoid stools for microscopy by means of a modified formal–ether concentration technique

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The stool concentration method employed at the MRC in Durban is the formal–ether (FE) concentration, which concentrates both helminth ova and amoebic cysts. The FE method requires a step whereafter the specimen is mixed with 10 % formalin, it is filtered through 2 layers of wet gauze, resulting finally in a fairly clean, small deposit for microscopy, after centrifugation. Concentrating fluid and mucoid stools has always been a problem as the parasites are removed with the filtrate when the stool is poured through the gauze, resulting in no findings in the deposit on microscopy. In January 2001 our laboratory undertook a provincial hospital-based parasitological survey in KwaZulu-Natal to determine, primarily, the prevalence of amoebiasis in the province, and secondarily to note the occurrence of other intestinal protozoal and helminth parasites. As the survey commenced we realised the concurrent cholera epidemic was going to impact detrimentally on the culture and microscopy outcomes of our study as more than 80 % of the stools received were diarrhoeic stools.

Total stools	A (watery fluid)	B (mucoid/fluid)	C (formed/semi-formed)
5733	3403 59.3 %	1292 22.5 %	1083 18.9 %

We therefore modified the FE method for the watery, fluid and mucoid stools as follows:

A) Watery and fluid stools: the specimens were spun down and the deposits examined microscopically.

B) The mucoid and mucoid/fluid stools were processed by the FE method, but were *not* filtered through gauze.

C) Normal formed and semi-formed stools were processed by the recommended FE method.

The modification to the concentration technique resulted in clumps of parasites being found embedded in the mucus in some stools, which would, with the normal FE, have been filtered off. Unfortunately, time constraints and limited specimen receiving volume prohibited the performance of a comparative study to accurately determine the extent of the improvement in parasite recovery resulting from the modifications. This improvement was, however, substantial and justified its employment in the study. *Entamoeba histolytica/E. dispar* is prevalent in approximately 10 % of the general population in Durban and its environs. Robinson's culture technique is employed in our laboratory to isolate these amoebae. Unfortunately, this culture method yields better growths when normal stools are inoculated. This method was also modified, as the amoebae in fluid/mucoid stools do not grow on inoculation into the first-day overlay. All abnormal stools (82 %) were therefore cultured into the second-day overlay, resulting in 26 growths, giving 0.5 % prevalence.

A comparison between the locally produced Polychem Kit and the imported Brazilian Kato-Katz Kit for use in monitoring helminth control programmes

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In 1997 a Parasite Control Programme was set up in Kwa-Zulu/Natal with the intention of using the imported and commonly used Brazilian Kato-Katz Cellophane Faecal Thick Smear (CFTS) Kits for monitoring purposes. A prerequisite was to train staff from the Health Department's 8 Health Regions to run their own test-and-treat campaigns for geohelminth infections and schistosomiasis. At

this time we were also provided with a locally made kit (Polychem Kato-Katz Kit). This, like the Brazilian kit that is distributed by the World Health Organisation, consists of both washable components that can be re-used for years and disposable items that can be bought separately. In order to compare the prevalences and intensities of geohelminth and bilharzia infections obtained using these 2 kits, we ran a trial at nearby Carrington Heights Junior Primary School. This was selected both for its proximity and because most scholars were from a nearby informal settlement where prevalence rates were known to be high. Three hundred and twelve children who obtained parental consent were tested over a period of 1–1½ months during February and March 1999. Single stool samples were taken under strict observation so as to prevent children sharing samples with friends who could not supply their own. Children who could not pass a stool at some time during the collection period were excluded from the study. The 312 samples were tested in small batches on the day of collection by the same technologist, making 1 Polychem CFTS and 1 Kato-Katz CFTS from each. A student did formal–ether concentrations on the same samples in order to check for protozoan parasites that are not detected by the CFTS, to examine diarrhoeal stools and to compare results with those of the CFTS. The 2 CFTS Kits gave similar results, but those from the formal ethers were (as expected) totally different. We concluded that the Polychem CFTS was very competitive in relation to accuracy and price, and should be considered as the standard kit for future control programmes in South Africa.

Teachers deworm children in a sustained programme in a west coast community

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Mass-deworming programmes where teachers have administered the anthelmintic tablets have been successful in Zanzibar, Ghana, Tanzania, India and Indonesia, but this has not been achieved in South Africa to date. In 1996, a committee of women from Langebaan North, a community on the West Coast of South Africa, approached the Medical Research Council about undertaking a nutritional status assessment because of concern about the physical condition of some children. Surveys of helminthic infections were included, because intestinal parasites can affect nutrition, growth and cognitive performance of children. During the baseline survey, 2 faecal samples per child were analysed. In subsequent surveys, 1 sample was examined. Stool specimens were processed using the formal–ether concentration technique and egg counts for intestinal helminthic infections were expressed quantitatively per 1 g of faeces. The baseline survey of the primary schoolchildren ($n = 234$) showed prevalences of 72.6 % for *Trichuris trichiura* and 18.4 % for *Ascaris lumbricoides*. The community decided to implement deworming of pre- and primary schoolchildren, and results were monitored in the latter. Teachers treated the children and kept treatment registers with help initially from researchers and support staff. During the research phase from August 1996 until November 1998, children were dewormed 3 times per year with 500 mg mebendazole (Vermox® Janssen-Cilag), subsidised by the manufacturer. Faecal samples were collected immediately before the next treatment so that the impact of the previous cycle could be assessed. The public health phase started in 1999 when the Local Authority took responsibility for the deworming programme. Teachers continued to treat the children twice a year and kept registers. They were assisted by a co-ordinator trained during the research phase. Generic mebendazole (D-Worm SD® Triomed) was used to minimise cost. As a result of 11 dewormings between August 1996 and November 2000, the prevalence of infection was reduced from 72.6 % to 14.0 % for *Trichuris* and from 18.4 % to 0 % for *Ascaris*. Arithmetic mean egg counts dropped from 2251 eggs per gram of stool (epg) to 25 epg for *Trichuris* and from 414 epg to 0 epg for *Ascaris*. Geometric mean egg counts decreased from 88 epg to 54 epg for *Trichuris* and from 60 epg to 0 epg for *Ascaris*. This is the first community in South Africa in which teachers have administered the deworming drugs and kept accurate treatment registers. This method is cost-effective and sustainable and is the only way to get the capacity required to deworm all children simultaneously. The procedure conforms to World Health Organisation recommendations.

A deworming project carried out by health personnel at primary schools in the Boland/Overberg Region

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The Integrated Nutrition Programme (INP) initiated assessment of the prevalence of infestation by intestinal parasites at schools targeted by the Primary Schools Nutrition Programme because these organisms can impair nutrition, as well as indicate disease risks linked to environmental pollution by human faecal material. The Medical Research Council (MRC), the Peninsula School Feeding Association, the Environmental Health Programme and the South African Wine Industries Trust contributed research, human and technical resources, funding and advice. In the first phase, a cohort of 44 randomly selected schools was established. After informed consent was obtained, 15 boys and 15 girls in Grade 3 were identified per school, again at random. Each child supplied a faecal sample and was measured to assess growth. Faecal analysis indicated that the prevalence of helminths exceeded 40 % at 23 schools. In these schools, 4446 children from pre-primary to Grade 4, were dewormed in October 2000. Treatment was supported by health education on how sanitation combined with personal and food hygiene can prevent exposure to infection. In order to complete this task, 13 environmental health officers (EHOs), 2 dietitians, 2 nutrition advisors and 2 school nurses, worked in teams. The EHOs also surveyed the drinking water, toilets and sanitation system. In 21 other schools where the prevalence of helminths was <40 %, only health education was provided to 4960 children in April 2001, by 9 EHOs, 1 dietitian and 1 school nurse. Follow-up deworming at the 23 schools took place in mid 2001. In response to positive feedback and requests from the communities, 7282 children in all grades were dewormed by 13 EHOs, 1 dietitian and 2 school nurses. A questionnaire was completed by all those who participated in deworming and health education, to assess the perceptions of the project by the targeted communities, school staff, and team members. The INP found that the intersectorial collaboration within the health services were highly successful. The EHOs perceived their involvement with deworming, as particularly beneficial because it strengthened their relationship with the communities, thereby facilitating all aspects of their work. The exercise has shown that a supportive relationship between environmental and community health can be achieved by this method. The EHOs have therefore initiated health education at schools that were not included in the project. Children with symptoms of worms are referred to clinics for deworming. We conclude that this project has shown that EHOs can implement mass-deworming of schoolchildren very successfully, and there are positive spin-offs. The method should be used at all primary schools in Boland/Overberg and can be recommended to other Health Regions. The deworming was cost-effective because the generic anthelmintic used cost less than 70 cents/treatment. Sustainability will depend on mobilisation of sufficient and appropriate human resources.

Deworming by teachers in Khayelitsha, near Cape Town, South Africa

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The World Health Organisation (WHO) has identified the control

of geohelminth infestation as a high priority for developing countries. The reasons include public, community and environmental health, as well as the morbidity and disease these worms can cause (Montresor *et al.*, 1999, WHO/CDS/CPC/SIP/99.3). When most of the children in a community are infected by the geohelminths *Ascaris lumbricoides* and *Trichuris trichiura*, it means that human faeces are polluting the environment. Amongst others, this indicates a lack of effective sanitation and high risk of epidemic enteric diseases caused by bacteria, viruses and protozoa. Sporadic deworming of individual children contributes nothing to overall control. Deworming at clinics cannot achieve synchronised treatment and children who do not need medical attention, should not be directed into clinics crowded by sick people. To initiate control of geohelminths by means of chemotherapy, pending more holistic measures, requires regular, synchronised deworming with effective, broad-spectrum anthelmintics. Since most of the burden of helminthiasis is in school-age children, pupils are optimal for implementing a treatment programme. The main obstacle is to obtain the human resource capacity to treat regularly and keep records. Health service personnel are too heavily committed elsewhere. In several developing countries, teachers deworm the children and appropriate health education is included in the curricula. This is in accordance with advocacy of health-promoting schools by the WHO. In South Africa, no generally acceptable and effective method of applying mass-deworming in schools has been achieved. At 12 primary schools in Sites B and C of Khayelitsha, where most of the people live in shacks, the communities and the educators have authorised and support a deworming programme. About 12 000 children have been treated regularly since 1999. Generic mebendazole (D-Worm SD®, 500 mg tablets, Triomed) has been effective against *Ascaris lumbricoides* and *Trichuris trichiura*. All batches of tablets used, are polymorph C mebendazole. The programme is cost-effective and sustainable.

Geohelminth transmission in slum-dwelling children in Durban, South Africa

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This prospective cohort study was conducted to determine the prevalence, intensity, incidence and re-infection rates of single and multiple geohelminth (*Ascaris lumbricoides, Trichuris trichiura* and *Necator americanus*) infections in 996 children aged 2–10 years living in 10 slums (informal settlements) in and around the Durban Metropolitan Area. Infection was assessed by microscopical examination of duplicate faecal specimens using a modified Kato-Katz technique, and intensity expressed as eggs per gram of faeces (epg) estimated from the mean of the 4 observations. A total of 15 308 samples was examined. A longitudinal study of patterns of re-infection with *A. lumbricoides* and *T. trichiura* was continued on 947 children following age-targeted chemotherapy with single doses of 400 mg albendazole. Two further cross-sectional studies, *i.e.* after periods of 4½–6 and 12 months, were conducted following treatment. Environmental risk factors which influence the transmission of geohelminths outside the host environment, *i.e.* climatic conditions such as relative humidity, temperature, soil type, altitude, slope and aspect were analysed for correlation with infection status between settlements. A quantifiable questionnaire was used to identify which risk factors (biological, environmental, socioeconomic and cultural) determine directly or indirectly the levels of infection among individuals. Prevalence and intensity of infections were very high and were compared among slums using Chi-square tests. Multivariate techniques were used to find which were the most important risk factors for infection/re-infection by each parasite. The models show that *A. lumbricoides* and *T. trichiura* are influenced by different risk factors. The relevance of this study is that it is one of only a very few studies on geohelminth transmission in urban slums and that the information provided on geohelminth epidemiology will be incorporated into a community-based control programme in KwaZulu-Natal.

A survey for intestinal parasites in faecal samples submitted to provincial laboratories in KwaZulu-Natal

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The importance of obtaining data on the prevalence of intestinal parasites has been re-emphasised by the potential impact of these infections on the pathogenesis of HIV/AIDS. In particular, data on true prevalence of *Entamoeba histolytica/E.dispar* has not been established, despite a recommendation that this is needed, made by a WHO/PAHO/UNESCO Expert Consultation (1997) after the redescription of the species in 1993. The objective of this study was to determine the prevalence of *E. histolytica/E. dispar* and other intestinal parasites among a patient population (children and adults) whose specimens were submitted to state laboratories in KwaZulu-Natal (KZN). From January to April 2001, 6313 faecal specimens (one/patient) were retrieved from 32 randomly selected laboratories in all 8 health regions of KZN. All specimens were analysed by microscopy following formol-ether concentration and were also cultured in Robinson's culture medium to optimise isolation of *E. histolytica/E.dispar*. One or more parasites were detected in 1233 of 5733 (21.5 %) specimens. Prevalence over the whole of KZN ranged between 0.03 % coccidian spp. and 10.69 % for *Ascaris*. The 2 helminth species *Ascaris lumbricoides* and *Trichuris trichiura* were the most prevalent (10.69 % and 6.7 % respectively). The prevalence levels for the other parasites were: *Entamoeba coli* (2.84 %), *Hookworm* (1.81 %), *Schistosoma mansoni* (1.03 %), *Entamoeba nana* (0.99 %), *Taenia* spp. (0.78 %), *Giardia lamblia* (0.72 %), *E. histolytica/dispar* (0.63 %), *E. hartmani* (0.47 %). The least detected parasites were *Chilomastix mesnili* (0.3 %), *Strongyloides* spp. (0.26 %), *Hymenolepis nana* (0.21 %), *S. haematobium* (0.19 %), *Enterobius vermicularis* and *Iodamoeba bueschtli* (0.12 %), *Isospora* and other coccidian spp. (0.03 %). Since the survey coincided with the recent cholera epidemic, the efficacy of parasite detection was compared between fluid and formed specimens.. The wide range of parasites found throughout the 8 health regions indicate a need for more rigorous studies to acquire accurate parasite prevalence data that will be useful to policymakers for appropriate intervention and control strategies.

An evaluation of a school-based programme for the prevention of helminthiasis in children

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This descriptive study investigated the implementation of a primary school programme designed to prevent parasitic infestation of children. The programme was piloted in 12 schools in Khayelitsha, Western Cape. Phase 1 involved the evaluation and monitoring of the training of master trainers (key educators whose task it was to train other educators). Phase 2 involved the evaluation and monitoring of the materials in the classroom. Interviews were conducted with educators from 6 of the 12 pilot schools in order to determine the factors that facilitated or hampered the success of the programme and to elicit ideas for the improvement. A semi-structured questionnaire aimed at supplementing the information gained from the interviews. It was completed either by key personnel or coordinators, at the 6 schools. Lessons were also observed in the classroom in order to determine the effectiveness and appropriateness of the materials. Factors that facilitated success, included active participation and involvement in the design of the program-

me, training by curriculum services, participation and involvement in a health-promoting schools group, the active involvement of the principal and support from colleagues. Factors that hampered success included competing curriculum priorities, lack of support from principal and colleagues, lack of basic facilities to monitor hygienic practices by children and conflicting messages from the community. Suggestions for the improvement of the programme included ideas around durability of materials, visual appeal of materials, ideas for improved learner interaction, classroom participation and involvement of parents and the community. This study therefore highlights the development of a school-based programme as part of an effective and sustainable prevention strategy in dealing with helminths or worms.

A sero-epidemiological survey of parasites in cattle in the northeastern Free State, South Africa

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Global economic losses due to parasitic infections in livestock are enormous. There is little or no information on parasites of veterinary importance afflicting livestock in the northeastern Free State, South Africa. A survey to determine the incidence of parasites in cattle (n = 386) was conducted in the northeastern Free State between August 1999 and July 2000. Giemsa-stained blood smears were negative for blood parasites. A total of 94 % of the cattle was sero-positive for *Babesia bigemina* by Indirect flourescent antibody test (IFAT) while 87 % were sero-positive for *Anaplasma* by enzymed-linked immunosorbent assay (ELISA). All the animals were sero-negative for *B. bovis* and this is probably because the tick vector (*Boophilus microplus*), which transmits the disease, is not present in the Free State province. There was no significant difference in the incidence of either anaplasmosis or babesiosis between the seasons. Two tick species belonging to the family Ixodidae, namely *Boophilus decoloratus* and *Rhipicephalus evertsi evertsi*, were found on cattle. In the present study significant differences in seasonal burdens of *B. decoloratus* occurred, with the highest infestations recorded from February to June. The presence of *R. evertsi evertsi* throughout the year without any, or with small, fluctuations in winter months was observed, with a peak from February to May. The observation of negative blood smears but high incidence of positive serological results for *Anaplasma* and *Babesia* for the same group of cattle indicates that this area is endemic for these diseases but with a stable disease situation.

Serological status of vaccinated and unvaccinated cattle to *Babesia bigemina* and *Babesia bovis* in an endemic tick-borne disease area of South Africa

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The serological status of cattle to *Babesia bigemina* and *Babesia bovis* was studied between August 2000 and June 2001, in the Limpopo Province of South Africa at Nooitgedacht ranch (24°33′S, 28°36′E), where calves were vaccinated against *B. bigemina* and *B. bovis*, and at Vlakplaas ranch (24°58′S and 28°05′E), where cattle had not been vaccinated against these parasites. The main objective of the study was to investigate whether there were significant serological status differences between vaccinated and unvaccinated groups to these redwater parasites in endemic areas. Seven-, 8-, 10-, 17- and 20-month-old cattle and breeding cows from both ranches were serum sampled and tested for the presence of antibodies against *B. bigemina* and *B. bovis* by the use of indirect fluorescent antibody test. Results indicate that cattle at Vlakplaas ranch had significantly higher serological status to *B. bigemina* than cattle of the same age group at Nooitgedacht. Only those cattle that had been vaccinated against *B. bovis* at Nooitgedacht ranch, were positive to *B. bovis* while all animals at Vlakplaas ranch were negative. It was concluded that *B. bovis* was absent from both ranches and the serological status differences between the cattle on both ranches were probably due to differences in the tick populations. Vlakplaas ranch, has been operating for 14 years, and has applied a relaxed tick control method. This probably ensured sufficient number of vector ticks for frequent transmission and maintenance of endemic stability to *B. bigemina*. At Nooitgedacht ranch, livestock farming was interrupted for about 3 years until 1999, and most likely had a lower tick population. Results at Vlakplaas ranch show that it may not be necessary to vaccinate calves against *B. bigemina* in ranches located in *B. bigemina* endemic areas and stocked with *Bos indicus* cattle or their crosses.

Diseases of free-ranging chickens in the Qwa-Qwa district of the Free State province of South Africa

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The study focused on determining disease-causing agents of free-ranging chickens in Qwa-Qwa, over a period of 6 months from June to November, 2000. In total, 177 chickens from 19 villages were bled from wing veins to prepare serum that was analysed by serological assays. ELISA, haemagglutination inhibition and rapid plate agglutination tests were conducted for infectious bronchitis, Newcastle disease and *Mycoplasma gallisepticum* infection respectively. Pooled fresh faecal samples from poultry houses were used to determine coccidia and helminth infections in birds using McMaster and Visser sieve techniques. Ectoparasites were collected from birds, nests and crevices and identified to genus level. Data from serological results indicate that 5 % of chickens tested were exposed to Newcastle disease, 88.8 % to infectious bronchitis and 63.1 % to *M. gallisepticum* infection. The helminth species isolated from faecal samples in 37 % of the villages investigated were *Heterakis*, *Ascaridia* and *Capillaria* species. *Eimeria* species, which is a coccidia, was also isolated 32 % of the villages investigated. The red fowl mite (*Dermanyssus gallinae*) was isolated from some of the birds and their nests in 16 % of the villages investigated. Questionnaire surveys were used to determine owners' knowledge and perspectives on poultry diseases. Only 10.5 % of owners interviewed had knowledge on poultry diseases. All the farmers reported that they had never received any technical support and that their chickens had never been vaccinated. There is an urgent need for veterinarians and animal health technicians together with the government to support free-ranging poultry farmers by providing subsidised vaccination in order to stimulate economic development in impoverished rural areas of South Africa.

Survival of *Paenibacillus larvae* larvae spores

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Paenibacillus larvae is the causative agent of American foulbrood of the honeybee *Apis mellifera*, and can infect humans. It is a Gram-positive bacterium that forms extremely resistant endospores. Only larval honeybees are infected. A parasitic mite, *Varroa jacobsoni* (*V. destructor*), which feeds on larval and adult bees, is a mechanical vector for *P. l. larvae*. Adult worker bees are also mechanical vectors because they inadvertently distribute the bacterial spores while tending to larvae. The aim of this investigation was to document the resistance of spores under realistic bee-keeping practices. Flamed and unflamed surfaces of equipment and hives, various outdoor bee drinking sites and bee faeces, were sampled with sterile swabs. Swabs were plated out on semi-selective MYPGP agar medium and incubated at 35 °C. Bacterial colonies identifiable as *P. l. larvae* were visible after 4 days. Results showed that all samples from drinking sites and bee faeces were negative. However, in a temperate climate, endospores on wooden and metal surfaces can resist outdoor ambient conditions for more that 2 years, and flaming of wooden surfaces does not guarantee killing of all spores. It was concluded that all contaminated wooden materials should be incinerated, and metal materials should be safe after thorough flaming.

The amphibian chytrid, *Batrachochytrium dendrobatidis* (Chytridiomycota: Chytidiomycetes) in South Africa

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Chytridiomycosis, a disease of amphibians caused by the chytrid fungus *Batrachochytrium dendrobatidis* has been responsible for many amphibians undergoing massive population reductions in Australasia, the Americas, and more recently in Europe. *B. dendrobatidis* has low host specificity and is likely to infect any species of amphibian as infections have been detected globally in 15 amphibian families that include 94 species. Amphibian chytridiomycosis is an emerging infectious disease of amphibians and has been recognised as such on a global scale. The purpose of this study is to determine whether the chytrid fungus from South Africa poses the same threat to our amphibian biodiversity as *B. dendrobatidis*, and to implement measures for lessening the risks. The fungus will be detected and cultured mainly from the host, *Xenopus laevis* (African clawed frog) in wild populations, museum collections as well as supplies from the international trade facilities. The chytrid fungus is diagnosed from the presence of zoosporangia in the epidermis of anuran skin. Histological sections of skin tissue are stained with haematoxylin & eosin and suspicious structures are identified with a more sensitive immunoperoxidase procedure. Cultured zoospores from various specimens of the chytrid fungus will be fixed in 100 % ethanol. DNA will be extracted from each sample, and PCR used to amplify the rDNA ITS (internal transcribe spacer) region. The fragment will be sequenced using an automated sequencer, and standard software will be used to align and compare the sequences. This technique will indicate if variation exists among populations. Three records of the amphibian chytrid have emerged from Africa from 1998 to 2000. In 1999 a batch of the tropical clawed frog, *Xenopus tropicalis* imported from West Africa into USA was found to be infected with chytridiomycosis, and in Kenya 2 individual plain grass frogs, *Ptychadena anchietae*, were found to be infected. The third occurrence was in a sample of *X. laevis* from the Western Cape, South Africa, in 2000. The chytrid had a prevalence of 60 %; however the frogs showed no clinical signs associated with chytridiomycosis. It is not known where *B. dendrobatidis* initially originated. The most likely hypothesis is that the chytrid escaped from an original host and locality, and has evolved as a pathogen in other environments and novel hosts species. The preliminary evidence supports the hypothesis that the host is possibly *Xenopus* spp. in Africa.

Malaria control in South Africa: an overview

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This paper gives a descriptive overview of the past, present and future trends in malaria control within South Africa. About 10 % of the total population of Spoth Africa live in malaria endemic areas (approximately 4 million people). The low-altitude areas of South Africa, namely Limpopo Province, Mpumalanga and KwaZulu-Natal (KZN) are endemic for malaria. Prior to 1985, the annual national malaria incidence was below 7500 cases. Episodes of outbreaks, each reporting 15 000 cases or fewer, occurred in 1985, 1987, 1988 and 1993. The number of malaria cases increased substantially to 27035 in 1996 and the number of deaths was the highest compared to the previous 25 years. The incidence has further increased, from 26 445 in 1997 to 61 934 in 2000. The increase in the malaria incidence has been attributed mainly to: 1. Improved case finding, especially in KZN. 2. Increase in rainfall and temperatures. 3. The movement of people carrying parasites across borders. 4. Increase in-parasite resistance to first line malaria drugs. 5. Mosquito resistance to insecticides. The National and Provincial Departments of Health in consultation with National Malaria Advisory Group (MAG) has developed the following strategy for the control of malaria in SA: (A) Improving case management of patients – this is achieved through prompt and correct diagnosis, coupled with appropriate treatment of malaria cases. (B) Planning and implementing selective and sustainable preventable measure, including vector control and usage of insecticide treated nets on the basis of malaria surveillance. (C) Detecting epidemics early and preventing and containing them. (D) Strengthening capacity to ensure sufficient and sustainable human resources are available to assess the malaria situation timeously. (E) Mobilising the communities by creating awareness. This is seen as critical for the control of malaria. The achievements of the control programme thus far have been: the introduction of a new drug (Coartemeter) into areas such as in KZN where resistance to first-line drugs such as chloroquine and sulphadoxine-pyrimethamine, has been reported; the re-introduction of the insecticide DDT for the spraying of houses after it was determined that the vector *Anopheles funestus* had reappeared and was resistant to existing insecticides such as pyrethroids; the strengthening of the inter-country collaboration on controlling malaria through the Lebombo Spatial Development Initiative. As a result of effective implementation of the malaria control strategies there has been a fall in the incidence of malaria in the current year, especially in KZN.

The protective role of IgM in African trypanosomiasis

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Trypanosoma brucei causes African trypanosomiasis, or sleeping sickness, in humans (*Trypanosoma brucei rhodesiense* and *T. b. gambiense*) and nagana in cattle (*T. b. brucei*). The disease has killed more than 300 000 people in the last 15 years and costs Africa R33 billion annually. *T. brucei* is an extracellular protozoan pathogen transmitted by the tsetse fly which switches its surface coat (variant surface glycoprotein – VSG) to evade the host immune response. IgM is a potent serum agglutinator and complement activator and is thought to be a protective component of host serum during infection. Here we investigate, using recently generated IgM gene-deficient mice, the protective role of IgM in African trypanosomiasis. Balb/C wild-type ($IgM^{+/+}$) and IgM gene-deficient ($IgM^{-/-}$) mice were infected with 5×10^2–5×10^5 pleomorphic *T. b. brucei* (AnTat 1.1E). The research undertaken has involved approximately 15 independent experiments (4–9 mice/group). Parasitaemia was determined on a haemacytometer and mortality data was recorded. The liver, lung and spleen were collected for pathological analysis. Serological responses were determined in whole-trypanosome and VSG-specific ELISAs (days 5–10). During this period we established *ex vivo* assays for trypagglutination and trypanosome killing. We then investigated if IgM could kill trypanosomes *in vivo* using specially engineered monomorphic trypanosomes (those with a fixed VSG coat). Infected $IgM^{+/+}$ and $IgM^{-/-}$ mice were challenged with monomorphic AnTat 1.1E and MiTat 1.6 *T. b. brucei* during the first peak of parasitaemia and mortality data was recorded. Parasitaemia control was impaired significantly in $IgM^{-/-}$ mice as compared to $IgM^{+/+}$ mice during the first peak (mean P-value <0.0001). Consequently, $IgM^{-/-}$ mice died more rapidly and exhibited more severe pathology than $IgM^{+/+}$ mice. Histopathological analysis revealed marked multifocal necrosis in the spleen and interstitial pneumonia in $IgM^{-/-}$ mice. Our *ex vivo* assay showed parasitaemia control during the first peak in $IgM^{+/+}$ mice was concomitant with trypagglutination and trypanosome killing, control mechanisms which were completely absent in $IgM^{-/-}$ mice. Serological responses (IgM, IgG2a, IgG3 and IgD) were typical of the infected host in $IgM^{+/+}$ mice whereas $IgM^{-/-}$ mice substituted the loss of IgM with dramatically elevated levels of IgD, while maintaining comparable levels of IgG2a and IgG3 to $IgM^{+/+}$ mice. Finally, we demonstrated unequivocably and consistent with our *ex vivo* observations of trypagglutination and trypanosome killing, that IgM, unlike IgG2a, IgG3 or IgD, effectively kills trypanosomes *in vivo* using the monomorphic challenge model. We demonstrate, using IgM gene-deficient mice that IgM, *via* trypagglutination and trypanosome killing, is the key effector immunoglobulin in parasitaemia control in African trypanosomiasis.

The role of IL-12 in African trypanosomiasis

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African trypanosomiasis has re-emerged to epidemic proportions. An agent of this disease *Trypanosoma brucei brucei*, is an exclusively extracellular pathogenic protozoan. It is thought that type 1 cytokines such as interleukin-12 (IL-12) and interferon-γ (IFN-γ) are important in protection against this parasite. IL-12 is a heterodimeric protein consisting of p35 and p40 subunits, together forming IL-12p70. IL-12 is important in specific immunity by promoting T helper 1 (Th1) cell differentiation leading to cellular immune responses. A predominant trypanosome-specific Th1 cytokine response is known to occur early in infection. In this study, mice deficient for IL-12p40 or IL-12p35 were compared to wild-type mice (WT) in their resistance to *T. b. brucei* infection. Comparative infection studies using IL-12-deficient mice and C57Bl/6 controls: mice (5 per group) were infected intraperitoneally with 5×10^3 parasites of the pleomorphic *T. b. brucei* AnTat 1.1E clone. Parasitaemia burden was determined using a haemocytometer; commencing 4 days post-infection. Serum was collected at day 13 post-infection for cytokine, chemokine and serological analysis. The date of mortality was recorded, and the liver, lung and spleen were collected for histopathological analysis. Mice deficient in IL-12 were found to be extremely susceptible to *T. b. brucei* with increased parasitemia and rapid mortality. Absence of IL-12 had no influence on efficient clearing of parasites during the first peak of parasitaemia but significantly increased parasitaemia thereafter. Immunoglobulin serum levels were rather normal, with elevated *T. b. brucei*-specific IgM, IgG 2a, and IgG 3 compared to WT mice. Increased IgG 1 profiles suggest a Th2 shift in the IL-12p35 KO mice. IFN-γ and TNF-α production was present in infected knockout mice, suggesting IL-12-independent endogenous production of those cytokines. *Post mortem* histological analysis revealed only slightly increased gross pathology in gene-deficient mouse groups. This study provides evidence for a protective role of IL-12 in African trypanosomiasis as demonstrated by increased parasitaemia and rapid mortality in IL-12-deficient mice. Absence of endogenous IL-12 had no effect on protective IgM responses but increased type 2 IgG 1 responses were found. These results suggest that IL-12 is not crucial for protective B cell antibody effector functions. Studies on the role of IL-12 in the activation of macrophage effector functions, *i.e.* Kupffer cells, Th1 and cytotoxic T cell responses are ongoing.

Species-specific polymerase chain reaction assay to identify members of the major African malaria vector *Anopheles funestus* and allied species

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One of the major vectors of malaria is *Anopheles funestus* Giles. This species belongs to a group of at least 8 species, of which *An. rivulorum* Leeson is the only other member that has been implicated in malaria transmission. *An. vaneedeni* Gillies & Coetzee, another member of the *An. funestus* group, has been experimentally infected in the laboratory, but to date has never been implicated as a vector in the field. Identification of members of the *An. funestus* group is a necessity in any malaria control programme. Identification has previously been done using either morphology or cytogenetics. Morphological identification is difficult due to the high mortality under insectary conditions as well as the fact that it is very time-consuming. Cytogenetics is a more rapid method that uses half gravid females. However, *An. vaneedeni* and *An. funestus* have homosequential banding patterns, which complicates identification. Species-specific PCR primers have been developed based on sequence variation found in the internal transcribed spacer region 2 (ITS2). The PCR is capable of distinguishing between 5 members of the *An. funestus* group: *An. funestus*, *An. vaneedeni*, *An. parensis* Gillies, *An. rivulorum* and *An. leesoni* Evans. Identification using the species-specific PCR is not dependent on any life stage of the mosquito and both male and female mosquitoes can be identified using this assay.

Control of experimental trypanosmiasis is dependent on infection-associated tumour necrosis factor (TNF) production and anti-trypanosome antibody induction, but does not require the presence of peripheral lymphoid organs and is independent of lymphotoxin-a induction

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African trypanosomes are the causative agent of human sleeping sickness and a number of livestock diseases. For several years, we have been investigating different mechanisms of immune and non-immune mediated mechanisms of trypanolysis and trypanosomiasis control. This work has recently resulted in unravelling the mechanism that is involved in the direct trypanolytic effect of the inflammatory cytokine tumor necrosis factor (TNF). In the course of this investigation, we have found that the pathway of TNF endocytosis/trypanolysis is initiated by the binding of the cytokine to a flagellar pocket exposed $GlnNAc_2$-Man_{5-9} carbohydrate moiety present on all variant-specific trypanosome surface glycoproteins (VSG). Lysis is mediated by an intralysosomal toxicity effect of TNF. We have also used the unique properties of single domain VHH camel antibodies to generate a carbohydrate specific antibody fragment that efficiently neutralises TNF-uptake and TNF-mediated trypanolysis. Finally, using TNF-deficient mice, we have shown that TNF is not only involved in parasitaemia control, but is moreover the main trigger of infection-associated pathology. In the present study, we have extended our analysis of cytokine-mediated trypanosomiasis control and immunopathology, using both Lymphotoxin-alpha-deficient (LT-$a^{-/-}$) and LT-$a^{-/-}$/TNF double-deficient mice. It is important that these mice have no detectable peripheral lymph nodes and lack germinal centre formation upon immune stimulation. Experimental infections were performed by intraperitoneal injection of 5000 living pleiomorphic *Trypanosoma brucei* AnTat 1.1E parasites. Parasitaemia and occurrence of anaemia was followed by microscopical analysis of blood taken at regular time intervals from the tip of the tail of the infected mice. At a 7-day interval period, individual mice were sacrificed and serum was collected for anti-trypanosome antibody titre determination and cytokine analysis. Spleens were analysed for the occurrence of splenomegaly. Infection-associated mortality was determined in groups of 10 mice. The results show that the absence of LT-a alters neither early parasitaemia development, nor infection-associated pathology. However, *T. brucei* infected LT-a-deficient mice exhibit a better control of late stage parasitaemia levels and have a prolonged life span that coincides with the appearance of increased chronic-stage anti-trypanosome IgM/IgG2a serum titres. Finally, using LT-$a^{-/-}$/TNF$^{-/-}$ double-deficient mice, we show that in these mice *T. brucei* infections are very well controlled, and that infection-induced pathology is minimised. Together, these findings show that while increased IgM/IgG2a anti-trypanosome antibody titres are generated in the absence of LT-a peripheral lymph nodes and germinal centre formation and correlate with improved parasitaemia control, TNF and not LT-a has a major impact on trypanosomiasis-associated immunopathology.

Evaluation of a rapid diagnostic method (ICT) as an alternative to microscopy for the diagnosis of malaria in the Limpopo Province

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Microscopic examination of blood smears remains the gold standard for malaria diagnosis, but is labour-intensive and requires skilled operators. Rapid diagnostic kits provide a potential alternative. A study was conducted in the Limpopo Province of South Africa, to evaluate the use of ICT, an immunochromatographic

antigen detection assay for the diagnosis of malaria using histidine-rich-protein-2 (HRP-2), as an alternative to microscopy in patients with suspected infections. Out of the 268 samples collected in this study, 55 and 54 samples tested positive when microscopy and the ICT method were used respectively. Moreover, of the 55 samples diagnosed microscopically, 4 were co-infected with *Plasmodium falciparum* and *P. malariae* and 1 with *P. falciparum* and *P. ovale* whereas of the 54 samples that tested positive with the ICT kit, 41 contained mixed infections of *P. falciparum* and *P. vivax*. The other positive samples were singly infected with *P. falciparum* using both methods. Our results indicated a lack of specificity and sensitivity when using the rapid kit procedure as compared to microscopy and as such this new kit cannot be reliably used for the diagnosis and differentiation of *P. falciparum* and *P. vivax* at a clinical level.

Molecular epidemiology of Malaria: implications of human migration for malaria resistance in South Africa

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Malaria, one of the killer diseases in Africa, is caused by *Plasmodium* species and transmitted by the female *Anopheles* mosquito. Treatment of all susceptible forms of malaria with chloroquine is now limited as a consequence of drug resistance. The antifolate drug combination Fansidar (pyrimethamine and sulfadoxine) is used as a first-line treatment for chloroquine resistant *P. falciparum* malaria patients. Unfortunately, resistance to this antifolate chemotherapy (Fansidar) has emerged and has been shown to result from point mutations in the dihydrofolate reductase (*dhfr*) and dihydropteroate synthetase (*dhps*) genes, which are targets for pyrimethamine and sulfadoxine respectively. Mutations in the *dhfr* gene at codons 59 and 108 are associated with resistance while additional polymorphism at codons 16, 51, 50, 140 and 164 results in increased resistance. Similarly mutations at codons 436, 437, 581 and 613 on the *dhps* gene correspond with resistance to sulfadoxine. The purpose of this study is to assess comparative relationships of malaria gene flow and migration by mapping *dhfr* and *dhps* mutations from isolates in South Africa and surrounding countries. Migration is one of the epidemiological factors that contribute to specific changes in infectious diseases. Major population shift across South Africa since political change of 1994 has resulted in people travelling to and from the rest of Africa where malaria is endemic. These cross-border movements and settlements have implications for malaria gene flow and resistance in South Africa.

Tetramethylpiperidine-substituted phenazines inhibit growth of *Plasmodium falciparum* by interfering with parasite haemoglobin metabolism

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Two derivatives of clofazimine (B633), B4119 and B4158 (1–8 μM) were evaluated for activity against chloroquine-, quinine- and sulfadoxine/pyrimethamine-sensitive and resistant strains of *Plasmodium falciparum in vitro* as well as for their effects on polymerisation of haeme to β-haematin. By using microscopic and flow cytometric methods, it was found that B4119 and B4158, but not clofazimine, inhibited growth of sensitive as well as resistant strains of *P. falciparum* with IC_{50} values between 0.22 and 0.7 μM, indicating a lack of cross-resistance. Inhibition of parasite growth was associated with interference with polymerisation of haeme to β-haematin *in vitro*. The data presented in this study demonstrate that the TMP-substitution at position 2 of the phenazine nucleus of riminophenazines confers anti-plasmodial activity of these compounds and they may prove useful forerunners in the design of novel anti-malarial pharmacological agents.

Abstracts of Posters

Unusual parasites and common artefacts found in human stools

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Parasitology laboratories are occasionally confronted with 'microscopic enigmas' for identification, in the form of unusual or spurious parasites and artefacts that often resemble parasites. This poster aims to give an insight into some of the uncommon parasites received for identification, and also to show some of the common artefacts that resemble parasites and to demonstrate the differences between them. All stool specimens were processed for microscopy by the formal–ether concentration method. Some of the photographs of 'parasites' demonstrate:

- Unidentified large ciliates found in 2 subjects, both living in Dundee, northern KwaZulu-Natal.
- A larva found in the concentration deposit.
- Artefacts resembling *Ascaris lumbricoides* eggs.
- Free-living *Strongyloides stercoralis* larvae.
- A spirurid ova.
- An artefact strongly resembling an adult *Ascaris lumbricoides* worm.

These findings, although not common, can be encountered in a diagnostic parasitology laboratory. It is therefore important to undertake very careful and thorough examination and appraisal of unusual specimens.

Trypanosoma clariense Pienaar, 1962 (Sarcomastigophora: Kinetoplastida) in *Clarias gariepinus* (Burchell, 1822) and its biological vector *Batracobdelloides tricarinata* (Blanchard, 1897) (Hirudinea: Glossiphoniidae)

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Previous surveys carried out in the Olifants and Vaal rivers has revealed that trypanosomes are abundant endoparasites of the sharptooth catfish, *Clarias gariepinus* (Burchell, 1822), in these river systems. Trypanosomes were absent from all specimens of *Barbus aeneus, B. kimberleyensis, Cyprinus carpio, Labeo rosae, L. umbratus,* or *Oreochromis mossambicus* collected from these rivers. *C. gariepinus*, infected with trypanosomes were kept in an aquarium, and leeches were allowed to feed on these fish. The leeches were then placed in a separate aquarium together with an uninfected, laboratory-reared catfish, which became infected by trypanosomes within 10 days after the leeches had fed. Intermediate stages of the trypanosome life cycle were also detected in serial sections of a leech. It is concluded that this leech is responsible for the transmission of a *Trypanosoma* sp. to *C. gariepinus*, and the leech is identified as *Batracobdelloides tricarinata* (Blanchard, 1897). The trypanosome-infecting *C. gariepinus,* which is host-specific to the catfish, is identified as *T. clariense* Pienaar, 1962. A technique using flow cytometry was devised in order to detect and quantify trypanosome infections in *C. gariepinus*. It was found that flow cytometry shows the presence of an infection, but that determining the intensity of infection is problematic and needs more specific attention.

A comparative study of stomach and intestine contents in *Barbus aeneus* and *Barbus kimberleyensis* in the Vaal Dam to clarify variance in tapeworm infestation

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A study of stomach and intestine contents in large-mouth yellow-

ish (*Barbus kimberleyensis* Gilchrist and Thompson 1913) and smallmouth yellowish (*Barbus aeneus* Burchell, 1822) was carried out during summer and autumn 2001 respectively. The study was carried out in the Vaal Dam in the Vaal River system. Stomach contents of adult specimens of both sexes were analysed macroscopically. Tapeworms were removed from digestive tracts, fixed, stained and identified. Total digestive tract length was measured and compared between the 2 species. Stomach contents in adult *B. kimberleyensis* consisted mainly of fish remains, while stomach contents of *B. aeneus* consisted mainly of plant material. Tapeworm infestation by the cestode *Bothriocephalus acheilognathi* (Yeh, 1955) was found in stomach and upper digestive tracts of both species, although prevalence in *B. kimberleyensis* was 93.75 % compared to 30 % in *B. aeneus*. The mean intensity reflected a similar pattern, *i.e.* 95.13 in *B. kimberleyensis* compared to 2.5 in *B. aeneus*. During summer all specimens collected from *B. aeneus* were immature but mature specimens were collected from this host in autumn. For a given length of fish, mean gut lengths were higher in *B. aeneus*. Dietary preferences correlate with gut anatomy, *i.e.* a shorter intestine is found in *B. kimberleyensis* which feeds mostly on fish and a longer intestine is found in the plant-material feeder *B. aeneus*. From the literature, metacestodes use planktonic or benthic copepods as intermediate hosts. This would make *B. aeneus* the preferred host. To the contrary, this study indicates that *B. kimberleyensis* is the preferred host. This phenomenon remains enigmatic.

The unique ambulacra specialisations of the louse fly *Pseudolynchia canariensis*

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Pseudolynchia canariensis is a common ectoparasite found on pigeons as well as on wild birds. Heavy infestations of this bloodsucking fly may cause aneamia in adult birds and the death by exsanguination of nestlings. These louse flies are also the main vectors of avian malaria. This study investigated the functional micro morphology that enables these parasites to attach to and move so rapidly through the feathers of their hosts. Live louse flies were collected from swifts and fixed directly in 70 % ethanol and ultrasonicated before dehydrating through graded ethanols, and critical-point dried. They were sputtercoated with carbon and gold before viewing in a Leica Stereoscan 420 scanning electron microscope at 5 to 7 kV. The first specialisation this study revealed was the pair of large compound ungues (claws) terminating each leg. Each claw consisted of 3 parallel blades with fine grooves for gripping the feather barbules. A pair of large pad-like pulvilli was found between each pair of claws. The ventral surface of each pad was made up of many fine processes that had bifurcated tips. The small globules observed on these tips may be adhesive droplets by which the pulvilli adhere to smooth traction surfaces. An empodium covered with many stiff setae projected medially between the pulvilli. This may be pushed into the feather held by the ungues, to anchor it more firmly. These specialisations are important to enable these parasites to remain firmly attached to their avian hosts, not only during grooming but also during flight.

The KwaZulu-Natal school-based parasite control programme: 1998–2000

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In 1998 the KwaZulu-Natal Department of Health initiated a school-based programme for the treatment and control of 3 species of soil-transmitted helminths and for schistosomiasis. The programme continues, and is intended to benefit the estimated 1.5 million children who attend primary schools in areas where clean water and sanitation are inadequate. The aim of the intervention is to reduce the prevalence and intensity of the target infections permanently, through a combination of treatment, health education and improved sanitation. The research presented here was commissioned by the Department of Health and describes the progress of the control programme from the baseline survey at the beginning of 1998 through to the end of the year 2000. The study area is the southernmost health region in the province. This region stretches from the mountains to the sea, with considerable variation in the environmental conditions thought to affect transmission. A 10 % random sample of schools was drawn from the 4000 primary schools that fitted the criteria for inclusion in the programme, with adjustments in the selection to take account of the size of the schools. In 1998, 5 pupils were randomly selected from each of the first 4 grades in these 40 schools, and their parasite and nutritional status, health knowledge, and school and domestic sanitation were monitored in annual surveys over the 3 years of the evaluation. Each year a further 5 pupils in each school were added to the surveillance sample from the new intake in Grade 1. The results show that overall the prevalence and intensity of the target infections dropped significantly during the 3-year pilot period, but that the available resources and the consequent success of the programme varied considerably across 4 altitude zones in the study area.

Serum cross-reactivity of *Ascaris*-infected Xhosa children to the fish parasite *Anisakis*

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Helminth infections are common in vast regions of the world and affect approximately a quarter of the world's population. Worldwide increasing allergies to a food-borne parasite, *Anisakis simple*x, have been linked to the ingestion of this nematode which can induce allergic reactions such as urticaria, angiodema and severe anaphylactic reaction but also infections of humans with live worms, causing anisakiasis. The infection rates of South African fish with *Anisakis pegreffii* are exceptional high, with close to 100 % in all species analysed. The aim of this study was to identify the frequency of *Anisakis* sensitivity in 750 South African children. The IgE reactivity to *Anisakis* and *Ascaris* was compared using radio-allergosorbent tests (CAP-RAST), RAST-inhibition assays and Western blotting. Skin prick tests (SPT) were performed on all 750 subjects using 6 common allergens and an in-house SPT of the local species *Anisakis pegreffii*. Over 60 % of the 750 analysed children had *Ascaris* infections and 55 were SPT-positive to *Anisakis*. Cross-reactivity was demonstrated by RAST-inhibition assays, suggesting that *Ascaris* is the primary sensitising antigen. RAST-inhibition and Western-blot studies demonstrate that *Anisakis* allergens are heat stable suggesting that accidental consumption of the fish parasite *Anisakis* may have implications for allergic reactions in *Ascaris*-sensitised children in South Africa.

IgM specific *Toxoplasma* antibodies among women attending public sector antenatal clinics in KwaZulu-Natal

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Toxoplasmosis is a disease of immunodeficient individuals (HIV infected) caused by the protozoan parasite *Toxoplasma gondi.* Infections with *T. gondi* could accelerate the progression to AIDS in HIV-infected individuals. Pregnant women are susceptible to infections by *T. gondi.* This places the fetus at risk of becoming infected through vertical transmission, resulting in congenital toxoplasmosis. The congenital form of toxoplasmosis is a serious disease that causes hydrocephalus, cerebral atrophy, blindness and mental retardation in newborns and children. The risk of pregnant women becoming infected and transmitting the parasite transplacentally is increased if

they are co-infected with HIV. Our study focused on acquiring baseline data for the prevalence of *T. gondi* infection among pregnant women attending public sector antenatal clinics in KwaZulu-Natal, South Africa. A cross-sectional observational study was conducted among HIV infected and uninfected women in Durban and surrounding areas. The indirect immunofluorescent antibody test was used to detect IgM specific *T. gondi* antibodies. Using standard statistical analysis the point prevalence of *T. gondi* infection was calculated. The overall prevalence of IgM specific *T. gondi* antibodies was 16.5 % (95 % CI: 14.7–18.5 %). In HIV positive women the prevalence was 14.5 % (95 % CI: 11.3–18.4 %) compared to the prevalence of 15.7 % (95 % CI: 13.6–18 %) in HIV negative individuals. The presence of dual infection (HIV and toxoplasmosis) in 14.5 % of women increases the risk of congenital toxoplasmosis. Future studies need to focus on the interaction between CD4 cell counts, *T. gondi* infections and pregnancy outcomes. This study should be of practical value to public health policy makers and clinicians when formulating guidelines for managing HIV and *T. gondi*. infected pregnant women.

Anasakis pegreffii: antigen recognition and antibody production in experimentally infected mice

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Almost all of fish in southern African waters are parasitised by the nematode *Anisakis pegreffii*, which can cause gastrointestinal and/or allergic reactions in humans after ingestion of raw, undercooked or pickled fish. Recently it has been shown that dead larvae and excretory products can also cause allergic reactions. However, the interaction between an immune response to infection and an allergic reaction is not well understood. A murine experimental model of anisakiasis was developed in BALB/c mice. Mice were infected orally and intraperitoneally with third stage larvae (L_3) of *A. pegreffii*. Three weeks after the initial infection, both groups were re-infected with live and dead nematodes. In addition, a group of mice was injected with L_3 protein extracts using alum as an adjuvant. The kinetics of the antibody response was analysed over a period of 3 months using enzyme-linked immunoassays (ELISAs). In addition the immune response was analysed by histology comprising identification of granulomas, determination of blood eosinophils in the jejenum and determination of Th1/2 cytokine response in spleen and lymph nodes using RT-PCR. Furthermore, the immune responses following the different infection methods were compared by Western blotting to identify the immunogenic proteins involved during an infection. Finally, the immune response in mice was compared to that of a human anisakiasis reference serum, and was found to display considerable similarities. The earliest antibody responses were found after approximately 1 week, with production of the IgM isotype. Throughout the infections, immunoglobulin production consisted primarily of IgM and IgG1, and a Th2 response was indicated by the cytokine pattern obtained. These findings suggest that the mouse is a useful model for studying the immunobiology of *A. pegreffii* in humans. Subsequent studies using gene-deficient mice will enlighten the regulatory role of cytokines in the protective host immune response and allergic reactions.

Treatment of acanthamoebic keratitis

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Acanthamoebic keratitis is being reported with increasing frequency in various parts of the world. The results of medical therapy for this serious ocular disease have, to date, been disappointing, and the optimal treatment regimen has yet to be defined. We tested the effects of drugs on isolates of *Acanthamoeba* from southern Africa and the United Kingdom. Amongst others, the cytopathogenicity of these strains and the effectiveness of contact lens disinfecting solutions on them, have also been studied (Niszl & Markus, 1998, *Br. J. Ophthalmol.* 82: 1033–1038; Niszl, Veale & Markus, 1998, *J. Parasitol.* 84: 961–967). Amoebae were axenically cultured and exposed to drugs in wells of microtitre plates. Viability of amoebae was assessed by their ability (or otherwise) to excyst on non-nutrient agar plates seeded with *Escherichia coli* bacteria. Polyhexamethylene biguanide (PHMB) and chlorhexidine (CHX) were effective against the cystic stage of all isolates. The effective range for PHMB (1.7–15.0 g/mℓ; mean 7.03 g/mℓ) was lower than that for CHX (7.6–18.3 g/mℓ; mean 13.72 g/mℓ). Both drugs are used at a concentration of 0.02 % for the treatment of acanthamoebic keratitis. This exceeds the respective ranges determined by us. We ascertained that of various drugs tested, high concentrations of the following killed cysts of a southern African strain of *Acanthamoeba*: mefloquine hydrochloride, natamycin and pentamidine isethionate. Amphotericin B, fluconazole, ketoconazole, miconazole, polymyxin B sulphate, propamidine isethionate and sulfisoxazole were ineffective at concentrations of greater than 1000 μ/mℓ. The most successful means of treating acanthamoebic keratitis so far, has proved to be a combination of a cationic antiseptic (CHX or PHMB) and propamidine isethionate. Our investigations confirm that in the present state of knowledge, this would normally be the best treatment for southern African cases of ocular acanthamoebic infection where drug sensitivity data are not available.

The efficacy of ivermectin against roundworms and ascarids in pigs in South Africa

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The study was designed to determine the anthelmintic efficacy of ivermectin (Virbamec, G2588) given by deep intra-muscular injection, against natural infections of nematode parasites in pigs. Fifteen young, clinically healthy pigs, not treated with anthelmintics, and with faecal worm egg counts positive for ascarids and other nematodes were used in the study. The control group consisted of 6 pigs. The treated group (n = 9) received ivermectin by deep intramuscular injection in the neck area, at the prescribed dose rate of 300 μg per kg live mass. Allocation of pigs to groups took place in a random manner. The total number and type of worms excreted daily by each pig from after treatment until slaughter, was determined. The number and type of gastrointestinal worms remaining in treated and untreated pigs, was determined at slaughter. The number of worms recovered from the treated group at slaughter was compared with the number of worms secreted by that group *ante mortem* according to the following formula: % Efficacy = (mt – mr)/mt × 100, where mt = total *ante mortem* worm burden of the treated pigs, and mr = total number of worms recovered from the treated pigs *post mortem*. The worms of the untreated group were recovered and counted to serve as proof that the expulsion of worms between treatment and slaughter was the result of the anthelmintic treatment and not due to self-cure as is often happens when pigs are moved to new surroundings. The trial animals were housed individually in single pens in a partly closed shed. The floor was made of concrete. The trial animals received pig growth meal at 2 kg per pig per day. Clean drinking water was freely available. The ivermectin test solution was 96.88 % effective against *Ascaris suum* and 99.6 % effective against *Oesophagostomum dentatum*. No worms were expelled by any of the untreated control pigs.

Pilot survey: intestinal parasite control in dogs and cats by owners in Gauteng

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A pilot survey was conducted amongst companion animal owners in the Gauteng province, South Africa to establish the following:

- Knowledge of owners on internal parasites.
- Treatment regimes followed by owners.
- Sources of consultation used by owners with regard to intestinal parasite control in pets.

Questionnaires were distributed at a private veterinary practice in Pretoria and a day-care centre for toddlers in Alberton. Out of a total number of 250 questionnaires distributed, 119 respondents returned completed forms (47.6 %), with 63 being female, 42 male and 14 where the sex of the respondents were not indicated. The respondents owned a total number of 252 dogs and 68 cats; 84.87 % of the respondents consult a veterinarian about deworming programmes for their pets, while 11.76 % and 10.08 % consult pharmacies and pet shops respectively. Information about deworming was obtained by some respondents from more than 1 source. Most owners only deworm their pets once a year. Veterinary practices are by far the most favourable source to obtain anthelmintics (81.15 %), while other sources include supermarkets (17.65 %) and pharmacies (13.45 %). Some respondents used more than 1 source for obtaining anthelmintics.

The anti-microbial properties of *Carica papaya*

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The pawpaw tree (*Carica papaya*, Caricaceae) is widely utilised for its medicinal properties. The seeds and skin are reported to have, amongst others, analgesic, antibiotic, antiseptic, digestive, hypotensive and laxative properties. The objective of this study was to determine the antimicrobial effect of pawpaw seed, leaf and flesh extracts. The inhibitory effects of the different Pawpaw extracts were tested against the fungi *Aspergillis niger,* the yeasts *Candida albicans* and *Cryptococcus neoformans,* and the bacteria *Escherichia coli, Pseudomonas aeroginosa, Enterrococcus faecalis, Staphylococcus aureus* and *Bacillus subtilis*. The inhibitory activities of the extracts on the fungi and bacteria were compared to the inhibitory activity of neomycin and nystatin, but found to be negative. A continuous line of human kidney (Graham) cells were maintained in the presence of 5 % CO_2. Toxicity assays of the extracts were performed by using the MTT toxicity assay and the ^{3}H-Thymidine incorporation technique and revealed no harmful effects. Continuous cultures of the erythrocytic stage of the *Plasmodium falciparum* chloroquine-resistant (FCR-3) and chloroquine-sensitive (3D7) strains were maintained. The *in vitro* parasite growth in the presence of the extracts was assessed using the ^{3}H-Hypoxanthine incorporation technique with chloroquine as a positive control. The radio-labelled DNA was harvested and counted with a γ-counter. The concentration required to inhibit 50 % parasite growth (IC_{50} values) were determined and used as an indication of the antimalarial activity of the extracts. Preliminary data suggests a strong antimalarial activity of the pawpaw leaves and some activity of the seeds. These data indicate that the pawpaw plant warrants further investigation regarding other antimicrobial uses.

Malaria mortality over time from 1994 to 1999 in rural KwaZulu-Natal

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Recent increases in malaria deaths became a great concern for both the malaria control and malaria research managers. In order to ensure that proper malaria control measures were equitably allocated, an assessment of the extent of this increase in malaria deaths was needed. However, this assessment was not possible due to a lack of reliable sources of malaria death data. The aim was to identify different sources of and collect malaria death data with the objectives of identifying different, and reliable sources of malaria death data and to analyse these data spatially according to subdistrict, age and gender, in order to identify the groups mostly affected. A retrospective review of records from hospitals, clinics, home affairs, community offices and police stations, in the districts of Ingwavuma, Ubombo and Empangeni in northern KwaZulu-Natal was undertaken. In addition, a community survey was planned to collect records of deaths that were not reported to these institutions. Results showed that only the hospitals kept malaria death data. None of the other identified possible sources could provide the required data. In total, 332 deaths due to malaria were recorded at the 4 hospitals, 161 females, 164 males and 7 unidentified as to gender. Of these deaths, 67 occurred at Bethesda, 99 at Manguzi, 147 at Mosvold and 19 at Ngwelezane. Only data for 1999 was found from Ngwelezane Hospital in Empangeni because records for the previous years had been destroyed to create more storage space. The community-based survey was not undertaken due to financial constraints. The collected death data are still being analysed to calculate case-fatality rates by hospital (or subdistrict), gender and age.

Workshop 1

Priming of T-helper cell differentiation may influence the outcome of infectious and parasitic diseases

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A major breakthrough in the understanding of immunity against infectious agents was the finding that polarised T helper (Th) cell differentiation into Th1 and Th2, regulated by interleukin (IL)-12 and IL-4, respectively, determines the outcome of the immune response to the causative organism. Th1-mediated responses (type 1) are mainly protective against intracellular bacterial, viral and protozoal infections, whereas Th2 responses (type 2) are host-protective in many helminth infections, but they are deleterious in allergic responses. Using gene-deficient mice, we have explored these relationships in several disease models, such as listeriosis, leishmaniasis, and helminthiasis, as well as in allergy. In this workshop, the consequence of T cell priming towards Th1 or Th2 on the outcome of co-infections as well as other diseases, were considered. Moreover, the questionable regulatory role of IL-4 in Th2 responses in *Leishmania major* and some other diseases were evaluated. Our research and that of others has established that Th1 responses, including interferon (IFN)- and IL-12, are crucial to innate and adaptive immunity in listeriosis (Dai *et al., J. Immunol*. 1997; 158: 5297. Brombacher *et al., Int. Immunol.* 1999; 11: 325). With the same model, it has also been shown that there is a positive feedback loop mediated by conventional T cells, which is an alternative mechanism for release of IFN-γ (Andersson *et al., J. Immunol*. 1998; 161: 5600. Bregenholt *et al., J. Immunol.* 2001; 166: 1871). By contrast, Th2 responses are crucial to resistance of helminthiasis, with IL-13 the major mediator of immunity to infestation by some helminths (Grünig *et al., Science* 1998; 282: 2261). *Leishmania major* is an intracellular protozoan pathogen. In mice it causes either acute or chronic disease. The Th2 cytokine IL-4 is associated with an acute inflammatory reaction that is potentially lethal in murine cutanous leishmaniasis. Paradoxically, the Th2 cytokine IL-13 has some protective function during chronic leishmaniasis (Mohrs *et al., J. Immunol.* 1999; 162: 7302*).* However, neither IL-4 nor IL-13 are necessary for Th2 differentiation during leishmaniasis (Mohrs *et al., Inf. Immunol.* 2000; 68: 1773). More research is necessary to elucidate how these Th2 immunological contrasts are mediated in murine leishmaniasis.

Impact of schistosomiasis on HIV-1 susceptibility and progression: HIV-1 chemokine co-receptor analysis and *in vitro* HIV-infectivity

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Increased levels of immune activation due to abundance of parasitic infections among persons in the developing world have been hypothesised to be partly responsible for the unique behaviour of the AIDS pandemic in these areas compared to the trend observed in more industrialised countries. Observations suggest that in general people in the developing world tend to be more susceptible to HIV infection, which once acquired, is followed by a relatively shorter period prior to the onset of AIDS. Though there are many

potential explanations for this faster progression to AIDS and death among HIV-1 positive persons in the developing world, one possible explanation is given by Bentwich *et al.* 1995. They hypothesised that the polarised Th2 responses and immune activation caused by chronic helminth infections that are abundant in sub-Saharan Africa provide a milieu that favours more rapid viral replication. We explored this possibility using schistosomiasis as a model of a chronic helminth infection. First, we evaluated what effects treatment of schistosomiasis would have on the circulating viral load of a person co-infected with schistosomiasis and HIV-1. For these patients, we did not observe an immediate reduction in HIV-1 RNA concentrations. We have also begun investigating the susceptibility of peripheral blood mononuclear cells from active or former schistosomiasis patients to *in vitro* infect ion with HIV-1. Cells are stimulated with antigen or mitogen exposed to monocytotropic or T cell tropic strains of clade A virus. Following 8 hours of infection, the virus is washed away and cells are recultured in the absence of additional stimulation. Supernatant aliquots are removed on successive days and tested for p24, indicating viral replication. Preliminary results suggest that cells from persons with active schistosomiasis may be more susceptible to viral infection than are cells from treated patients. Similarly, cells from persons with active schistosomiasis had higher levels of the chemokine receptors CXCR4 and CCR5 expressed on the surface of their CD4 cells. These preliminary data are consistent with the hypothesis that helminth infections may increase susceptibility to infection with HIV-1 upon exposure to virus.

Ascariasis suppresses the immune response to an oral vaccine

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Geohelminth parasites infect a large proportion of the world's population and *Ascaris lumbricoides* is the most prevalent of all helminth infections. We have hypothesised that *A. lumbricoides* infection in the small intestine may affect the immune response to oral vaccines and partly explain the relatively poor immunogenicity of several live oral vaccines reported in Africa, Asia and Latin America. We conducted a randomised controlled study in rural Ecuador in which *Ascaris*-infected children were randomised to receive either albendazole or placebo followed by a single dose of the live attenuated oral cholera vaccine CVD 103-HgR. Rates of seroconversion of vibriocidal antibodies were greater in the albendazole-treated group compared to the placebo group (29.3 % *vs* 15.6 %, $p = 0.06$). Pre- and post-vaccination cytokine responses to cholera toxin B-subunit (CT-B) were examined in a sample of the albendazole and placebo-treated children. There was evidence of a suppression of interleukin-2 responses to CT-B in those children with active ascariasis (placebo) compared to those treated with albendazole, and interferon-gamma responses were suppressed in both groups compared to a group of uninfected controls. The data provide evidence that concurrent ascariasis is able to suppress antibody (vibriocidal) and cellular (antitoxic) responses to CVD 103-HgR, and that eradication of infection with courses of anthelmintics treatment can reverse part of the suppressive effect.

Interaction of BCG vaccination scar status and Mantoux skin test responses with ascariasis and trichuriasis in a community with a high incidence of tuberculosis

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[BCG = Bacille Calmette Gurin, ARTI = annual risk of TB infection, TB = tuberculosis, IgE = immunoglobulin E.]

BCG vaccination and infection with *Mycobacterium tuberculosis* are known to elicit a type 1 immune response, while intestinal helminths are often associated with a type 2 immune response. The epidemiology of tuberculosis and intestinal helminthic infection were studied in 2 adjacent suburbs of Cape Town that resemble a third world setting. The TB notification rate for this community is 1000/100 000 per year and the *ARTI* is 3.5 %. The mean serum *IgE* level for adults is >300 kU/l. During the *ARTI*-survey, the *BCG* vaccination scar status, the Mantoux skin test responses and the helminth burden of all primary school children (5–15 years) in the community were recorded. The study now reported, sought to determine whether children with or without a *BCG* vaccination scar were infected by *Ascaris lumbricoides* and/or *Trichuris trichiura,* and the possible effect of these parasites on the Mantoux skin test response. A total of 2616 children were enrolled and were examined for a *BCG* scar, parasites and Mantoux skin test response. Only 64 % of the children had a positive *BCG* scar and 53 % were infected with *Ascaris lumbricoides* and/or *Trichuris trichiura*. A positive correlation was found between Mantoux positivity and the presence of parasites ($P < 0.0001$). The presence of a *BCG* scar (RR = 0.972, $P = 0.078$) had no effect on the size of a Mantoux response, whereas age (RR = 1.652, $P < 0.0001$) and the presence of parasites (RR = 1.303, $P = 0.0005$) were significant factors influencing the outcome of the Mantoux response. Children with a positive *BCG* scar had a significantly reduced prevalence of *Trichuris* (RR = 0.92, $P = 0.008$) and *Ascaris* (RR = 0.92, $P = 0.023$) infection. These associations remained after correcting for age, sex, area (TB incidence) and HAZ (growth). The results are difficult to explain immunologically as they show that (1) children with a *BCG* scar had a reduced prevalence of helminths, and (2) children with a positive Mantoux response had more helminths. This may have far-reaching implications with regard to vaccine development for regions such as Africa where parasites, TB and HIV/AIDS are particularly prevalent.

Cell-mediated immune responses in rural Ugandans co-infected with the filarial nematode *Onchocerca volvulus* and the *Human Immunodeficiency Virus* (HIV) type 1

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Uganda is involved in HIV vaccine trials in adults and anticipates paediatric HIV vaccination. Analysis of cell-mediated immune responses in Ugandans is therefore necessary. Early and constant exposure to helminths skews the T-helper (Th) cell profile to a type 2, known to cross-regulate type 1 responses that are fundamental in controlling viral infections. Furthermore, anthelmintic chemotherapy can influence Th cell responses. The study was done, in the Kabarole District of western Uganda, to determine T lymphocyte responses in co-infection with *Onchocerca volvulus* (Ov) and the HIV. Differences between individuals with and without microfilariae (mf) following 4 years of 12-monthly treatment with Ivermectin, a microfilaricide that also leads to expulsion of intestinal nematodes, were evaluated. Peripheral blood mononuclear cells derived from young people were cultured with and without phytohaemagglutinin, tuberculin (PPD), tetanus toxoid (TT), Ov worm extract or *Candida albicans* antigen. Cytokine release in cell-culture supernatants was determined by sandwich ELISA. Lymphoproliferation was quantified by incorporation of thymidine. In Ov-HIV co-infection, spontaneous interleukin-5 (IL-5) release was high and significantly increased in mf carriers, while interferon-gamma (IFN-γ) was hardly detectable. In the same group IL-5 and IFN-γ production to parasite-specific antigen was extremely low. Lymphoproliferation in response to Ov antigen and TT, was increased in mf-negative and HIV-negative, Ov-infection, but not in mf-negative Ov-HIV co-infection. Proliferative response to PPD was slightly reduced in Ov-infection and significantly reduced in Ov-HIV co-infection. Th2 immune responses predominate in young Ugandans constantly exposed to gut and tissue helminths. Co-infection with HIV augments the immune activation, resulting in impaired response on antigenic challenge. Regular deworming resulting in parasite clearance may prevent the dominance of type-2 immune responses, reduce immune activation and alleviate cell energy.

The impact of *Mycobacterium tuberculosis* and gut parasite co-infections on the status of HIV-1 infection: an observation cohort study

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The incidence of tuberculosis (TB) in South Africa is approximately 528 TB cases/100000 per annum, which is over 60 times worse that in the USA or Western Europe. TB remains the leading cause of morbidity and mortality and coupled with the high prevalence of HIV-1 infection, represents a formidable challenge for considering treatment options. With this in mind, knowledge of the interactions of HIV-1 and TB at the epidemiological, clinical and immunological levels are required to facilitate the type and duration of possible therapeutic and preventative strategies. A further complexity in these interactions is the confounding factor of gut parasite infections, where >50 % of individuals with TB or HIV-1 infection would require treatment for a variety of worms. We set out to examine cohorts of HIV-1 and TB single and co-infected patients admitted to King George V Hospital, Durban, where 73 % of newly diagnosed TB patients were HIV positive. One of our aims was to investigate the effect of gut parasite burden on clinical features and response of TB and TB/HIV-1 co-infected patients treated with standard TB therapy. To date, we have recruited 95 newly diagnosed TB patients, where 69 patients were co-infected with HIV-1. Various proportions of these patients were also co-infected with gut parasites, so that 4 groups could be delineated: TB; TB plus parasites; TB/HIV; TB/HIV plus parasites. The effects of these different co-infections on clinical features, HIV-1 burden and T cell immunocompetence will be presented. The impact of these different co-infections on immunity is important to understand so that strategies towards HIV or TB vaccine development can be formulated.

School-based eosinophilia can be reduced by mass-deworming

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Helminthic infection is the main cause of eosinophilia in developing countries (Rothenberg M E, *New Engl. J. Med.*, 1998; 338: 1592–1600). However, eosinophil counts have apparently not been monitored during school-based deworming programmes. Mean eosinophil counts of pupils at 2 primary schools in the Western Cape exceeded the upper limit of the normal paediatric range ($0.45 \times 10^9/\ell$).Regular treatment with mebendazole at 1 school and with albendazole at the other, at intervals of 4 months, resulted in reduction of mean counts to within the normal range ($P < 0.05$). The greatest reductions were in the highest counts. Recruitment and activation of eosinophils is an effector mechanism of humoral immunity and is mediated by T-helper 2 (Th2) lymphocytes and their cytokines. Chronic Th2 activation can downregulate the Th1 immune responses that are needed to combat HIV/AIDS and tuberculosis, including immunisation. In conclusion, control of helminthiasis by mass-deworming, together with implementation of effective sanitation, hygiene, problem-orientated health education and alleviation of poverty, has potential to influence immunological responses to co-endemic diseases.

Workshop 2

Parasitology and commercial game farming in South Africa

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Since the 1960s, there has been a steady increase in the numbers of game farms in South Africa and they now occupy significant portions of commercial agricultural land. In the Limpopo Province, game farms had covered a total of 3.6 million ha by August 1998. By comparison, the Kruger National Park, South Africa's prime ecotourism destination, covers approximately 1.9 million ha. It has been reported that despite the increase in the size of this industry, the ecological management of game ranches is generally not scientifically based and lacks quantitative monitoring. Farms are often overstocked, animals are introduced into unsuitable habitats and species that do not naturally occur together are placed into the same areas. These factors lead to an increase in stress, a degree of immune suppression and an increase in parasite burdens in game animals. Hosts carrying large numbers of adult ticks are probably the most readily noticeable signs of a problem on a game farm and the measurement of parasite burdens is considered a valuable parameter of farm management practices. The growth in popularity of wildlife ranching is increasing the contact between domestic stock and indigenous wildlife. This can result in an increased exposure of exotic (domesticated) livestock to indigenous disease-producing organisms including diseases such as corridor disease, African swine fever, African horse sickness and heartwater. On the other hand indigenous wild animals are exposed more and more to exotic diseases that have the potential to produce severe effects in relatively naive populations. As the number of commercial game farms has multiplied, so has the demand for economically valuable animal species. This is illustrated by the high prices that are paid for 'disease free' buffalo. As a result, programmes have been established to breed buffalo free of the diseases for which they are hosts, including corridor disease, which is transmitted to domestic stock by *Rhipicephalus appendiculatus*. As the game farming industry increases, so also the importance of parasites that may be found in or on game. Parasite control using pesticides in wildlife is, to a large extent, impractical and costly. This calls for creative and innovative approaches to deal with the problem in free-ranging wild populations. A balanced approach is needed in which parasite control is based on sound management practices.

Some helminths of wildlife

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Most wild animals are distributed according to more or less well-defined geographic regions. Within these regions different species will have different preferences, that is, occupy specific niches. Thus, gemsbok prefer arid environments, impalas and gazelles a savanna type, and bushbuck and nyala frequent riverine forest. The host preferences will contribute towards the helminth species composition and their numerical magnitude. In large ecosystems such as the Kruger National Park, which is largely free of human interference, parasites (and predators) play a role in the selection of the strongest animals in a population. Normally, healthy animals harbour several thousand helminths, many of which may still be in an immature stage, and these large burdens seem not to adversely affect the hosts. Young animals are often subject to large helminth burdens and the weaker ones or those that do not develop an effective immune reaction against the helminths are removed from the population. Likewise, old animals whose immune system has become compromised, are removed. Maladaptation, disease, injury or stress causes helminth burdens to increase, which in turn weakens the animal further and hastens its removal from the environment. Predators are important for regulating both the prey population and the tapeworm population. The cysticerci that occur in the prey species must be consumed by the predator in order to close the life cycle, and where predators are absent, the continued existence of these parasites may be jeopardised. The author's own observations are that antelope have more trichostrongylids (direct life cycle), equids and pigs more strongylids (direct life cycle), primates and carnivores have spirurids (indirect life cycle), most groups have a sprinkling of ascarids (special life cycles), all have lungworms (*Dictyocaulus*, *Metastrongylus*, *Protostrongylus*, *Pneumostrongylus*, *Troglostrongylus*) (indirect life cycle) and hookworms (special life cycle). However, there are certain helminths, especially those that enter the host through the skin, and the ascarids that have adapted their life cycles in such a manner that infection through the milk or *via* the placenta (from mother to offspring) is possible.

Strongyloides is one that has both a free-living and a parasitic life cycle, males only occurring in the free-living cycle. It appears that the parasites are adept at recognising what their hosts eat and adapt their life cycle accordingly, and this is particularly evident in the cestodes of carnivores. Many cysticerci occur in the abdominal cavity on the mesenterium, which is the favoured food of especially lions and leopards. For this reason, these carnivores often share the same species of cestodes. Others are more host-specific, *i.e.*, the cysticerci of *Taenia olngojinei* that occur only in the pelvic bones of some gazelles and alcelaphines in Tanzania. Hyaenas are the only animals that can consume these bones, and the parasites are thus ensured of their life cycle continuing. The helminths of African wildlife remains a vast subject and with so many orders of mammals, birds, reptiles, amphibians and fish that occur on the continent, the possibilities are endless, as is the amount of basic research that has still to be done before applied research can commence.

The efficacy of amprolium against coccidiosis in captive buffalo (*Syncerus caffer*) in the Kruger National Park

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African buffalo (*Sincerus caffer*) are captured in Kruger National Park, South Africa, for a disease-free breeding programme. Pregnant females are captured and tested for tuberculosis and brucellosis; negative females are brought to the Skukuza Research area and are kept in captivity in bomas until calves are born. Calves are then, reared free of tuberculosis, brucellosis, foot-and-mouth and corridor diseases. During the time that the animals are kept in bomas, the confinement and increased level of stress present perfect conditions for coccidiosis to develop. Frequent cases of coccidiosis were found and some young calves subsequently died. The difficulties of developing a non-intrusive, anti-coccidial programme where the dosage of medication could be controlled, resulted in the use of amprolium in the drinking water. Faecal samples from the boma floors were collected and coccidial counts performed daily and weekly on individual and pooled samples to determine the base level of coccidial infection. Identification of some of the species was attempted and some species were found to be more abundant than others. Water containers were calibrated to determine the daily water consumption of the buffaloes and the dosage was decided accordingly. Monitoring of the coccidial infections was done on a daily basis and the consistency of the faeces and presence of clinical signs were also observed. Three groups of buffalo were selected and randomly allocated to a negative control group, a preventive group (amprolium 30 % 5 mg/kg/day for 21 days) and a curative treatment group (amprolium 30 % 10 mg/kg/day for 5 days). We conclude that both preventive and curative treatment seems to control coccidiosis in captive buffalo. More trials are being conducted.

Blood parasites affecting the game industry

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During recent years the game industry has become an important source of income for South African through hunting, tourism and the export trade. There are, however, blood parasites affecting the wildlife industry, causing considerable economic losses. Theileriosis can often be fatal in sable antelope (*Hippotragus niger*), tsessebe (*Damaliscus lunatus*) and roan (*Hippotragus equinus*) especially when these animals are stressed during capture and translocation. Theileriosis can also cause mortalities in other game such as eland (*Taurotragus oryx*) and giraffe (*Giraffa camelopardalis*). *Cowdria* has been reported to be fatal in springbok (*Antidorcas marsupialis*) when these animals are translocated to heartwater-endemic areas. *Anaplasma* infection occurs naturally in a wide range of wild ruminant species but is known to cause serious clinical disease only in the giraffe. Sable antelope are also susceptible to various *Babesia* species. It is important in the translocation of wildlife that 'new diseases' are not introduced into a susceptible population or into areas where such diseases are unknown but where they potentially could become established (*i.e.* when suitable vectors are present). This could lead to serious losses in susceptible populations. Therefore much research needs to be done and has been done to determine the susceptibility of various wildlife species to blood parasites and their ability to successfully carry and transmit these diseases.