Final programme
&
Book of abstracts

‘Dutch Parasitology is looking ahead...’
Congress in honor of 50 years
the Netherlands Society for Parasitology

Hotel Mooirivier, Dalfsen, the Netherlands
26th & 27th of May 2011
Welcome

On behalf of the Netherlands Society for Parasitology (Nederlandse Vereniging voor Parasitologie - NVP) we give you a warm welcome in Dalfsen.

We are very pleased to see you here as a participant of the congress ‘Dutch Parasitology is looking ahead...’ and to celebrate our 50th anniversary together with you.

In this book you will find the final outline of the congress and all the summaries of the invited speakers as well as the abstract presentations.

The Congress Committee has done its utmost to compose an attractive programme. We hope you will enjoy the programme and your stay in Hotel Mooirivier!

Prof.dr. Robert Sauerwein,  
chair of the NVP

Dr. Peter J. De Vries,  
chair of the Congress Committee
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<td>Elias van der Plicht (history researcher at the Nationaal Archief and journalist/writer)</td>
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<td>‘The Moses of Malaria. N.H. Swellengrebel abroad and at home, 1885-1970’</td>
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<td>Maiza Campos Ponce (Dept. of Health Sciences, VU University Amsterdam) 'Deworming improves asthma and temporarily deteriorates atopy: longitudinal anthelminthic treatment study in Cuba'</td>
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<td>Mihai Netea (Radboud University Medical Center Nijmegen) 'Pattern recognition of fungal pathogens'</td>
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<td>Wim Hendrikx</td>
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<td>(<a href="http://www.unionsalsa.nl">www.unionsalsa.nl</a>)</td>
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## Friday 27th of May 2011

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<td>Time</td>
<td>Session 4: Veterinary parasitology Chair: Joke van der Giessen</td>
<td>Katsuhiro Takumi (National Institute for Public Health and the Environment, RIVM) ‘Echinococcus multilocularis: the arrival to increasing public health risk in The Netherlands’</td>
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<td>Deborah van Doorn (Dept. of Infectious Diseases and Immunology, Fac. of Veterinary Medicine, Utrecht University) ‘In vitro selection for ivermectin resistance within four historically different anthelmintic treated cyathostomin larval populations’</td>
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<td>Louis Maes (University of Antwerp) ‘Drug resistance in Leishmania parasites’</td>
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<td>Vincent Delespaux (Institute of Tropical Medicine, Belgium) ‘Fire at will on drug resistant trypanosomes’</td>
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| 17.30-17.45 | Closing session of the congress          | Robert Sauerwein (Chair of the NVP)  
Peter de Vries (Chair of the Congress Committee) |
| 17.45-18.30 | Farewell drinks in the Holland Zaal      | End of the congress                                                              |
THE FIGHT AGAINST THE MOSQUITO. WALCHEREN 1919-1924

Elias van der Plicht
Freelance history researcher and journalist

Endemic malaria occurred in the Netherlands until the 1950s. The coastal areas were regularly plagued with the disease. In the twentieth century, Zeeland for instance dealt three times with a malaria epidemic: around 1900, between 1919 and 1924, and during World War II. The island of Walcheren was continuously the centre of the epidemics in the Dutch delta. During the epidemic between 1919 and 1924 the city of Middelburg was considered the centre of the disease. The epidemic resulted in a revival of malaria research, centred around the fight against adult mosquitoes. After the discoveries of Laveran, Ross and Grassi, late nineteenth century, a search began for the best control methods of the disease. During the malaria epidemic in Zeeland around 1920, there was plenty of experimenting with methods to tackle the mosquito in order to make malaria disappear. The Chief Inspector of Health, Terburgh, wanted to launch an anti-mosquito campaign in Zeeland. He wanted to know how effective such a campaign would be and whether by systematically controlling adult mosquitoes malaria could disappear.

In the winter of 1921-1922 a mosquito eradication campaign was launched on Walcheren. Although the mosquito population on Walcheren decreased, the anti-malaria campaign was no success: too many mosquitoes survived. In addition, a number of questions remained unanswered. It was not clear what percentage of the mosquitoes were eradicated. Besides, it was not sure whether the decrease in the number of mosquitoes was caused by the eradication campaign, or by natural causes. To better understand the extent to which the mosquito control was effective, it was decided to start another mosquito eradication campaign the following winter.

In the summer of 1923 it was concluded that the second winter
mosquito eradication campaign had again not been very successful. The number of mosquitoes was reduced, but there were still too many mosquitoes alive. There was dissatisfaction about the lack of major breakthroughs in the fight against the mosquito. Moreover, politicians threw doubt upon whether the measures were necessary. In addition to this, all went not well for the Netherlands financially. At various departments significant cutbacks were announced. Politicians questioned whether the fight against malaria could continue with fewer financial resources. In the meantime, there was some bickering about who should contribute to the fight against malaria. The first mosquito eradication campaign in the winter of 1921-1922 was entirely financed by the Central Government. It was agreed that in the years that followed, the province and the municipalities would contribute as well, but both the province of Zeeland and the municipalities failed to do this. According to Terburgh this should not mean that the destruction of the mosquitoes should cease. Although the Department of Health took a financial step back, a third mosquito eradication campaign was started in the winter of 1923-1924. Figures on a decrease in the number of mosquitoes following the third mosquito campaign have not been traced. It is clear however, that after the campaign of 1923-1924 the number of malaria cases declined dramatically. If malaria disappeared in 1924 because of the control measures or by natural causes, is a question that cannot be answered. At that time, there was no consensus about the causes of the disappearance of the disease. Now, eighty, ninety years later we know much more about the fight against malaria. But the pre- eminent solution has still not been found. A quote from the Member of Parliament Kersten is still very topical. In 1927, three years after the end of the malaria epidemic in Zeeland, he spoke the following words: ‘Humanity is far from omniscient. Why has malaria disappeared in Zeeland? Learned men have been asking this question so many times in recent years, but a satisfactory answer can still not be found. They are still searching malaria!’
HISTORY OF QUININE PRODUCTION IN THE NETHERLANDS

Cees K.W. van Veldhuizen
ACE Pharmaceuticals B.V // ARTECEF B.V., Zeewolde, The Netherlands

The Amsterdamsche Chinine Fabriek was worldwide famous for their extraction of Quinine from the Cinchona bark. The method of production of the different Quinine and Quinidine salts was located for more than a century in the centre of The Netherlands, in Maarssen.

Although Quinine lost and looses its importance for the treatment of malaria, Quinine still is in use in other applications. German competitors like Boehringer also focused on the promising possibilities of Quinine and derivatives.

For ACF the agriculture was highly important; even cellculture has been explored as possible solution for shortening the time to harvest as well as to enlarge the percentages of quinine in the bark to harvest.

In the eighties of the last century ACF shifted its attention to Artemisinin (KINA 2000). As a result DSM took over the Quinine extractionplant in Maarssen.

Surprisingly enough, there is still a shortage of Quinine in the world.

A secret will be revealed during the presentation.
HETEROGENEITY IN MALARIA TRANSMISSION: THE KEY TO SUCCESSFUL MALARIA CONTROL?

Teun Bousema  
*London School of Hygiene & Tropical Medicine*

The risk of malaria is not evenly distributed within malaria endemic countries, between villages or even within individual villages. Some individuals may experience several malaria attacks per year while others remain malaria-free for several years. The factors underlying this heterogeneity in malaria exposure are poorly understood and include environmental, household and human factors. Inter-individual variation in exposure to malaria and malaria-transmitting mosquitoes has consequences for disease transmission that can become 2- to 4-fold more efficient in comparison to a more equal distribution of exposure. Heterogeneity in malaria transmission is becoming increasingly important now the intensity of malaria is declining across sub Saharan Africa. Malaria elimination efforts on Zanzibar and Bioko are hindered by micro-epidemiological strongholds of malaria transmission that persist despite an overall decline in the burden of malaria. Understanding exposure patterns is crucial for the interpretation of epidemiological studies and the evaluation of malaria interventions. However, there is currently no consensus on how to detect hotspots of malaria transmission. Remote sensing approaches currently have limitations for detecting small-scale variation in malaria transmission. An approach that uses serological markers of malaria exposure may have a higher sensitivity at sub-village level and could provide an operationally attractive alternative. Recent findings from Mali, Kenya, Uganda and Tanzania will be discussed in the context of future plans to interrupt malaria transmission by targeting malaria hotspots.
MALARIA DIAGNOSTIC TESTING AND TREATMENT PRACTICES IN THREE DIFFERENT *PLASMODIUM FALCIPARUM* TRANSMISSION SETTINGS IN TANZANIA: BEFORE AND AFTER A GOVERNMENT POLICY CHANGE

Guido JH Bastiaens, Erik Schaftenaar, Arnold Ndaro, Monique Keuter, Teun Bousema, Seif A Shekalaghe
Nijmegen Institute for International Health, Radboud University; Nijmegen Medical Centre, Nijmegen, the Netherlands; Department of Community Health, Kilimanjaro Christian Medical Centre, Moshi, Tanzania; Department of Immunology & Infection; Faculty of Infectious & Tropical Diseases, London School of Hygiene & Tropical Medicine, London, UK; Kilimanjaro Christian Medical College, Kilimanjaro, Tanzania; Ifakara Health Institute, Bagamoyo Research and Training Centre, Tanzania

ABSTRACT

**Background**

Patterns of decreasing malaria transmission intensity make presumptive treatment of malaria an unjustifiable approach in many African settings. The controlled use of anti-malarials after laboratory confirmed diagnosis is preferable in low endemic areas. Diagnosis may be facilitated by malaria rapid diagnostic tests (RDTs). In this study, the impact of a government policy change, comprising the provision of RDTs and advice to restrict anti-malarial treatment to RDT-positive individuals, was assessed by describing diagnostic behaviour and treatment decision-making in febrile outpatients <10 years of age in three hospitals in the Kagera and Mwanza Region in northern Tanzania.

**Methods**

Prospective data from Biharamulo and Rubya Designated District Hospital (DDH) were collected before and after policy change, in Sumve DDH no new policy was implemented. Diagnosis of malaria was confirmed by RDT; transmission intensity was evaluated by a serological marker of malaria exposure in hospital attendees.
Results
Prior to policy change, there was no evident association between the actual level of transmission intensity and drug-prescribing behaviour. After policy change, there was a substantial decrease in anti-malarial prescription and an increase in prescription of antibiotics. The proportion of parasite-negative individuals who received anti-malarials decreased from 89.1% (244/274) to 38.7% (46/119) in Biharamulo and from 76.9% (190/247) to 10.0% (48/479) in Rubya after policy change.

Conclusion
This study shows that an official policy change, where RDTs were provided and healthcare providers were advised to adhere to RDT results in prescribing drugs can be followed by more rational drug-prescribing behaviour. The current findings are promising for improving treatment policy in Tanzanian hospitals.
Immunosuppression by *S. mansoni* reduces the protective immunity by radiation attenuated *P. berghei* malaria sporozoites.


*Department of Medical Microbiology, Radboud University Nijmegen Medical Centre. #Department of Parasitology, Leiden University Medical Centre.

**Introduction:** Whole parasite immunization may be an approach to develop an effective malaria vaccine since induction of sterile protection in human volunteers has been shown in experimental clinical settings. In nature, malaria parasites often co-exist in individuals chronically infected with other pathogens. Global *Schistosoma mansoni* distribution shows a geographical coverage that highly overlaps with malaria endemic areas. Moreover, some studies show that both parasites influence the natural course of each infection in co-infected individuals. The current study aimed to evaluate the protective capacity and immune response of *P. berghei* whole parasite immunizations in *S. mansoni* infected mice.

**Methods:** Uninfected and *S. mansoni* infected C57BL6/j mice were immunized by radiation attenuated *P. berghei* sporozoites (RAS) and challenged with wild-type sporozoites. Induction of both cellular memory and regulatory T cells (Tregs) was assessed by flow cytometry as well as cellular IFNγ responses after *in vitro* stimulation. *P. berghei*-specific antibody levels were measured by ELISA.

**Results:** RAS immunized *S. mansoni* infected and uninfected mice both showed increased CD4+ and CD8+ memory T cells in both liver and spleen. In these organs however, the *S. mansoni* infected mice showed higher levels of Tregs and lower IFNγ response by CD8+ memory T cells upon *in vitro* stimulation with *P. berghei*. 
sporozoites. In addition, these mice showed reduced protection by RAS. Interestingly, *P. berghei* specific antibody levels did not differ between the groups.

**Conclusion**: We provide evidence for immunosuppression by *S. mansoni* infection during *P. berghei* RAS immunization which associates with decreased malaria protective immunity.
IN VIVO IMAGING OF PARASITES: WHAT DO WE HAVE TO LOOK FORWARD TO?

Blandine Franke-Fayard  
Leiden Malaria Research Group, Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands

In vivo imaging tools offer a wide range of novel applications to study parasite-host interactions. Bioluminescence imaging (BLI) has emerged as a powerful method to study infectious diseases in animal models. Pathogens or mice are engineered to functionally express transgenic luciferase enzymes that are derived from bacteria, insects (firefly) or marine organisms. The decisive study showing the feasibility of detecting microbial generated luminescence within a living mouse in real time was published by Contag and colleagues in 1995, using Salmonella typhimurium transformed with a plasmid conferring constitutive expression of a bacterial luciferase (Photorhabdus luminescens). This work was the driving force that leads to additional studies on infection by bioluminescent Gram-negative and Gram-positive bacteria and viruses. More recently, a number of eukaryotic pathogens including, Plasmodium, Leishmania, Toxoplasma and Trypanosoma have been transformed with luciferase; this work uncovered unique insights into interactions of these parasites in real time and within their hosts. We at the Leiden Malaria Research Group are using the rodent malaria model of Plasmodium berghei to generate a range of transgenic bio-fluorescent reporter parasite lines, to aid in delineating the detailed host-parasite interaction at the cellular level and also help to uncover new anti-malarial therapies; this is achieved by using the ‘reporter’ parasites in combination with in vivo imaging to study aspects of malaria patho-biology, including the dynamics of sequestration of P. berghei infected red blood cells (irbc) in different organs in live mice. We have confirmed that P. berghei sequestration is analogous to that observed in the human parasite, P. falciparum; in that irbc containing the maturing forms (schizonts) are not present in the
peripheral blood but are sequestered in organs such as the lungs and adipose tissue. Moreover, our work has shown that *P. berghei* irbc adhere to the class II scavenger receptor, CD36, which is also one of the major human receptors to which *P. falciparum* irbc adhere. We are now exploring the possibilities of using *in vivo* imaging in conjunction with ‘falciparumized’ *P. berghei* parasites in mice that express the human receptor(s), in an effort to create a sensitive and biologically relevant *in vivo* screening system for testing chemo- and immunotherapies that interfere with *P. falciparum* sequestration, and in so reduce severe pathology.

Quantitative analysis of liver-stage development had been hampered both by the low levels of parasite infection in the liver and sensitivity of reporter gene detection technology; this resulted in difficulties in examining the dynamic interplay between the parasite and the host and also did not allow for easy quantification of intervention strategies. We have now applied *in vivo* imaging technology to detect bioluminescent *P. berghei* parasites through the mosquito stage and to the liver stage infection. With the increased sensitivity of the *in vivo* imaging system we are now able to visualize and discreetly examine single infected hepatocytes within livers of live mice. We are currently developing this methodology to validate targeted therapies against liver infection. We envisage that both the range of organisms and the feasibility to dissect the detailed molecular and cellular interactions by using BLI will continue to develop, yielding important insights into mechanisms of pathogenesis and so aid in the development of novel therapies.
ELIMINATION OF TROPICAL WORM INFECTIONS: ALSO A CHALLENGE FOR MODELLING

Wilma A. Stolk, Sake J. de Vlas AND Nico Nagelkerke
Department of Public Health, Erasmus MC, University Medical Center Rotterdam, Rotterdam, the Netherlands; Department of Community Medicine, United Arab Emirates University, Al Ain, United Arab Emirates

The current successes of international programmes to control tropical worm infections with mass treatment have led to great optimism that these once widespread diseases will eventually be eliminated. This applies to onchocerciasis (river blindness), lymphatic filariasis (elephantiasis), and – in some parts of the world – schistosomiasis (bilharzia).

These parasitic diseases have a number of things in common. They are chronic in nature, due to the long life span of parasite (several years) and repeated re-infection. Pathological changes develop slowly and accumulate until irreversible clinical manifestations become overt. Transmission is determined by individual intensities of infection, heterogeneities in behaviour, and complex vector-related factors. Regular mass treatment reduces disease progression in treated individuals and/or transmission of infection to others.

Ongoing elimination programmes face important practical questions. Typical questions are: how many treatment rounds are needed to achieve elimination? What is the optimal interval between treatments? How to determine whether treatment can safely be stopped and how to organize post-treatment surveillance? Empirical data for answering these questions are still scarce. Simulation models are useful tools for the evaluation of infectious disease control programmes and are also used to study research questions related to elimination.

The answers to these questions partly depend on local conditions. Elimination prospects are strongly determined by characteristics of the infectious agent (and human response to the infection) and
local transmission conditions. In general, the areas which are worst affected before start of treatment will require more intensive or longer duration of mass treatment. The required duration of mass treatment further depends on treatment coverage, but also on the amount of systematic non-compliance. Individuals who are systematically not reached by the control programmes can maintain transmission in the area and jeopardize elimination programmes. Another problem for elimination is posed by migration of infected humans or vectors from other areas: the infection can be re-introduced from outside. This phenomenon has hardly been studied. The importance of this depends on whether or not immigrating humans and vectors come from “controlled” areas or not and whether the migrating humans are effectively treated. The challenge for control programmes is to know where extra efforts are needed, to maximize the coverage and prevent systematic non-compliance, and prevent re-introduction of disease from outside. Another challenge to control programmes is to determine when treatment can stop. Proofing absence of transmission is difficult, because it is impossible to study everyone (especially in these low resource areas) and because diagnostic tests typically become less sensitive when infection intensity is low. Things are particularly problematic if the persons that systematically miss treatment also do not show up in population surveys for remaining infection. Low intensity infections hardly cause any symptoms, so that residual infection cannot be identified via passive case detection. Better diagnostic tests may be needed (such as immunological tests for measuring exposure or pool screening of vectors). Available models do not always take account of the issues raised here and need to be improved. Best suited for addressing elimination questions are individual-based models, which simulate all individuals in a population separately, taking account of village and individual characteristics.
Helminths have been shown to modulate the immune response which has been suggested to be essential for their survival in the host. For some helminths, this immunomodulation leads to the suppression of other immunopathologies such as allergy and autoimmune diseases. Part of this regulatory network include the regulatory T (Treg) cells, which are a subset of lymphocytes that play a key role in maintaining immune homeostasis by suppressing the immune response. *Trichinella spiralis* is a zoonotic helminth that infects a wide range of mammalian hosts. The larvae are released in the stomach, migrate to the small intestine where they maturate into adult worms and release newborn larvae that rapidly disseminate throughout the host, and eventually enter skeletal muscle to establish and remain in the host for many years. We have recently shown that *T. spiralis* antigens suppress dendritic cell (DC) activation in-vitro. In this study we aim at characterizing T cell activation induced by *T. spiralis*. For this purpose, DC pulsed with *T. spiralis* excretory secretory (TspES) antigen were incubated with spleen cells from OVA-specific TCR transgenic D011.10 mice and OVA. Cytokine production and the expression of T cell surface markers were measured. Results indicate that TspES-pulsed DC expand the CD4+CD25+FOXP3+ population compared to control cells. In addition, we found that TspES induce CD4+CD25+ T-cells that strongly suppressed ConA-induced proliferation of CD4+CD25-effector T cells compared to control cells. Infection of BALB/c mice
with T. spiralis resulted also in increased number of suppressive CD4+CD25+ T cells. Our results indicate that T. spirals induces both in vitro and in vivo, functional T regulatory cells.
DEWORMING IMPROVES ASTHMA AND TEMPORARILY DETERIORATES ATOPY: LONGITUDINAL ANTHELMINTHIC TREATMENT STUDY IN CUBA

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Introduction
Soil-transmitted helminth (STH) infections have been suggested to protect from atopy and atopic diseases, although there is still no consensus on their relationship. However if the relationship is true, anthelminthic treatment would increase the prevalence of atopic disease in STH endemic populations. We investigated the effect of deworming and STH (re)infections on atopy, asthma, allergic rhinoconjunctivitis and atopic dermatitis.

Methods
We examined 389 Cuban schoolchildren aged 4-13 in six-monthly intervals for 24 months. STH infections were diagnosed by stool examination. Atopic diseases were diagnosed by International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire and atopy by skin prick testing (SPT). STH infections were treated with one single dose of 500 mg mebendazole at every measurement period.

Results
After deworming the frequency of asthma significantly decreased (P<0.001). The percentage of SPT positives temporarily increased
from 10.2% (95%CI 4.5-15.9%) to 34.0% (95%CI 24.8-43.1%) and subsequently nearly returned to baseline values (14.4%, 95%CI 7.4-21.4%). (Re)infection with *A. lumbricoides* and *T. trichiura* was positively and hookworm negatively associated with having atopic diseases, while for atopy an opposite trend was seen.

**Conclusion**

Our results indicate that atopic diseases improve after anthelminthic treatment while atopy increases. As this increase appears only temporarily, anthelminthic treatment does not seem to be a risk factor for the development of atopy, nor for atopic disease. Effects of STH (re)infections on atopy and atopic diseases appear to be species-specific.
Recognition of fungal pathogens

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Candida species are the most common cause of fungal infections affecting either body surfaces (mucocutaneous infections) or the bloodstream and deep organs (candidemia and invasive candidiasis). Recognition of Candida by the cells of the immune system may result either in rapid elimination of the pathogen in the immunocompetent host, or the persistence of the pathogen in immunocompromised patients. There is good evidence that the genetic make-up of the host is important for the susceptibility to infections in general, and to fungal infections in particular. Recent research shed light on the recognition mechanisms responsible for the stimulation of the host defence by the fungus, as well as the innate genetic defects that may specifically predispose patients to inborn clinical syndromes associated with fungal infections such as chronic mucocutaneous candidiasis (CMC), hyperimmunoglobulinemia E syndrome (HIES), the dectin-1 deficiency and CARD9 deficiency. An overview of the host defence mechanisms and the known genetic factors influencing disseminated candidiasis, with an emphasis on Toll-like receptors, will also be presented.
MEDICAL TOURISM

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For centuries man has been travelling in a quest for better health and wellbeing. In more recent history travelling for medical treatment abroad has grown to an unprecedented scale because of air travel and internet. Although cultural and linguistic links with a foreign region and prior experience may encourage seeking care abroad, the major factors for seeking treatment abroad are quality and costs. Four different categories of patient mobility across borders can be defined: 1) primary exit; 2) duplicative exit; 3) institutionalized exit and 4) complementary exit.

Primary exit is “driven by escalation of out-of-pocket spending for health care and insurance premiums beyond the grasp of low- and middle-incomes”. This kind of patient mobility is more common in the US for the poor and under-insured.

The duplicative exit is more common in Europe and is driven by the search for higher quality or the avoid waiting lists. When formal programs and policies are in place to regulate patient mobility one speaks of institutionalized exit.

When a traveller goes abroad to take advantage of lower costs for dental care, reproductive care, cosmetic surgery, joint replacements, cardiac by-passes or transplantation this is called complementary exit. Many of these services are not covered or require substantial coinsurance. In several low- and middle income countries such as India and Thailand this complementary exit has created a new medical industry trying to attract travellers from high-income countries by offering cheap medical treatment in a luxurious holiday setting.

Medical tourism is not without risks. The risk of nosocomial infection
in low- and middle-income countries is much higher than in high-income countries. Infections with hepatitis B virus, multi-resistant Gram-negatives and methicillin resistant Staphylococcus aureus are but a few to name. Patients have little legal resources in the event a complication occurs or if the outcome of surgery is not what was desired for.

Finally, there are also ethical concerns. In general, medical tourism may divert resources away from the government run health services. In transplant tourism has been associated with significant ethical concerns such as swindling by organ vendors of payment organ donors receive, substandard organ procurement, and the use of organs from executed prisoners. Medical tourism is rapidly evolving and likely to continue to do so in the future.
WHAT’S NEW ABOUT MALARIAVACCINS?

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The most effective way to reduce death and disease from malaria will be administration of an effective vaccine to susceptible populations. Induction of long-lived immunity to *Plasmodium falciparum* (*Pf*), however, is a major obstacle to malaria vaccine development. Immunity to malaria is considered hard to acquire and artificial induction of sterile protection in humans has until now only been achieved by inoculating radiation-attenuated sporozoites through >1000 infective mosquito bites. We recently developed a protocol that demonstrates that sterile protection can be induced markedly more efficiently by inoculation of intact sporozoites under cover of a bloodstage anti-malarial drug. We further showed that protection is mediated by T cells eliminating liver parasites and antibodies targeting blood stage parasites. Cellular responses against *Pf* parasites, in particular IFNγ production, play an important role in anti-malarial immunity. *In vitro* cytokine (IFNγ, IL-2) responses were measured by flow-cytometry prior to, during and over a year post-infection. We show that cellular responses against both sporozoites and *Pf* infected red blood cells are readily induced and remain virtually undiminished at least 14 months after even a single malaria episode. These observations indicate that anti-malarial immunity can be induced more readily than previously thought and support the concept of whole-parasite-based malaria vaccines.
SCHISTOSOMIASIS: THE GOOD AND THE BAD

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Schistosomiasis affects more than 200 million people worldwide and although can be clinically silent in a large proportion of infected subjects living in endemic areas, in a subset and in travelers infected for the first time, it can lead to clinical complications as a result of immunopathology. Despite the absence of overt clinical manifestations in infected communities, it has become clear that a number of hematological and nutritional alterations lead to considerable morbidity, indicating the importance of developing vaccines against this highly prevalent parasitic infection. However, over the years, it has also become clear that there is a negative association between schistosome infections and inflammatory diseases such as allergies or autoimmunities. This has formed the starting point for research into regulatory mechanisms induced by schistosomes with the hope of identifying single molecules with immune modulatory properties that can be taken into clinical practice.

Studying an infectious disease requires sensitive and specific diagnostic methods. Improvement of diagnosis and its field applicability has put Dutch parasitologists at the forefront of schistosomiasis research. Continuous application of new methods, such as mass spectrometry or polymerase chain reaction to the diagnosis of schistosomiasis holds promises for the refinement of the identification of subsets of individuals not only with respect to infection status but also stage of pathology.

With enhanced diagnostic methods, the studies of the immune
status of infected populations improve. In search of vaccines, the intense epidemiologic studies whereby it has been established that “resistant” and “susceptible” groups of individuals exist has provided an important starting point for the dissection of immune responses to schistosome antigens. Not only the conventional approaches to identify proteins but alternative possibilities of finding glycans as possible vaccine candidates are now possible due to the development of new glycoimmunological tools by Dutch scientists. These new technological possibilities and “out of the box” thinking has been instrumental for Dutch parasitologists to take the lead in an EU funded program to search for a vaccine against schistosomiasis.

With respect to the schistosome mediated immune modulation, research on the understanding of host-pathogen interaction at the molecular level, has brought a number of Dutch parasitologists together to take on the immense task of characterizing the immunological network on the one hand and parasite signature molecules that drive such immunological networks on the other. Population studies in Gabon and Ghana, investigations in human cells in vitro, as well as animal models of schistosome infections have been combined with the in depth biochemical analysis of parasite molecules. Molecules have been identified that drive regulatory cells as well as effector Th2 cells. These molecules place Dutch parasitologists in a unique position that will allow the dissection of fundamental mechanisms underlying immune skewing; and just as important, their application to preventing inflammatory diseases.
A GLIMPSE OF SLEEPING MALARIA LIVER STAGE PARASITES

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Plasmodium vivax is the second important human malaria parasite that constitutes 25-40% of the ~515 million annual malaria cases worldwide in tropical climates, also extending into temperate climate zones. Although mortality rates of P. vivax infections are far less than from P. falciparum (malaria tropica), severe disease is quite common and hampers socio-economic development of regions where vivax malaria is endemic. P. vivax, as one of few malaria parasites that infect humans (P. vivax and P. ovale) and macaque monkeys (P. cynomolgi, P. fieldi, P. simiovale), forms dormant liver stages -hypnozoites- following sporozoite infection that can re-activate through unknown mechanisms to give rise to new symptomatic blood stage infection without re-infection. This dormant reservoir of parasites is an extra complication in efforts to eradicate malaria. Currently only one drug, primaquine, is known to kill hypnozoites and resistance has already been reported. Therefore, new drugs to eliminate dormant malaria parasites are needed and knowledge of the biology of hypnozoites would enhance drug development.

Given that P. vivax blood stage parasites cannot be cultured in vitro and thus heterogeneous parasite populations derived from human infections are the major source for parasites, we have developed the P. cynomolgi-rhesus monkey system as a reliable model system for human P. vivax malaria. P. cynomolgi blood stages can also not be cultured in vitro, thus primates are needed to provide infected
blood for ex vivo feeding of anopheles mosquitoes. Two weeks after feeding salivary gland sporozoites can be isolated for in vitro infection of primary hepatocytes. In ten days of hepatocyte culture, we can distinguish two parasite populations. One that is developing through to mature schizonts with first merozoites appearing around day 9 and one that grows out to 3-day old trophozoites and remain like that throughout the culture period. Drug-sensitivity profiles suggest that the latter population are hypnozoites as they are insensitive to atovaquone treatment (while liver schizonts die) but can be killed with primaquine. We are currently using this in vitro hepatic stage culture to evaluate new compound activity against hypnozoites and developing liver stages of P. cynomolgi. To begin to study hypnozoite biology, e.g. by proteomic analysis of parasites, we have generated transgenic P. cynomolgi that expresses both GFP and mCherry throughout the life cycle, including the hepatic stages. This now opens ways to isolate different populations of liver stage parasites for further studies.
EVERYTHING YOU WOULD LIKE TO KNOW ON BLASTOCYSTIS

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*Blastocystis* is the most common, non-fungal eukaryotic organism in the intestinal tract of humans and a vast array of non-human hosts. The genus comprises at least 13 morphologically identical subtypes, most of which have been found in humans. Each subtype is possibly equivalent to a separate species. Humans are mostly colonised by ST3 and ST1, followed in prevalence by ST2 and ST4. Although associated with irritable bowel syndrome, the clinical significance of colonisation with *Blastocystis* is not entirely clear, and it is hypothesised that differences in clinical outcome of colonisation is associated with differences in subtype. This presentation focusses on the identification of *Blastocystis* carriers and the applications and cave-ats of subtyping tools. Moreover data on the prevalence of *Blastocystis* and *Blastocystis* sp. subtypes in different Danish cohorts will be presented. Preliminary data from ongoing multilocus sequence typing (MLST) studies will be presented and the applicability and utility of MLST will be addressed.
TRENDS AND CHALLENGES IN DIAGNOSTIC PROCEDURES IN HIGH-INCOME COUNTRIES

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The recent introduction of DNA detection procedures, especially realtime PCR, has caused a strong shift in the organization of routine diagnosis of parasitic infections in many West-European countries. Driven by the need for laboratory automation, large scale testing and traceability of diagnostic outcomes, a growing number of laboratories even tend to expel traditional microscopy and replace this by DNA detection methods. However, despite the tremendous reduction in labor and the proven high sensitivity and specificity of real-time PCR, detection of parasite specific DNA also has its constraints. By definition the amount of targets is limited, genetic mutations and emerging new pathogens will be missed. Furthermore, due to the high sensitivity additional cases may be detected without complete understanding of the clinical relevance of these so called parasite carriers and their contribution in the transmission of disease.

In this brief presentation the pros and cons of different diagnostic procedures will be discussed. Should indeed molecular diagnosis replace microscopy, with the possible risk of further waning of local expertise in parasite morphology? Will there still be a niche for immunodiagnostic tests such as antibody detection and (copro)antigen assays? And what will be the future role of clinical parasitologists within a diagnostic work-up? Suggestions will be made how to continue to provide optimal quality in diagnostic
consultation in conjunction with the patient’s clinical data, covering a broad scale of parasitic diseases and patients with different backgrounds, including travelers and immuno-incompetent individuals.

Further background reading
PROTECTION AGAINST DIARRHOEA ASSOCIATED WITH ASYMPTOMATIC GIARDIASIS IS LOST WITH MULTI-NUTRIENT SUPPLEMENTATION: A PROSPECTIVE STUDY AMONG RURAL TANZANIAN CHILDREN

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Background: Asymptomatic infections with Giardia lamblia are common among children in developing countries, but the role of giardiasis as cause of diarrhea in such settings has been questioned. Impaired linear growth and cognition have been associated with giardiasis, presumably mediated by malabsorption of nutrients.

Aim: In a prospective cohort study, we aim to compare rates of diarrhea in pre-school children with and without G lamblia infection. In addition we assessed how micronutrient supplementation influenced the relationship between G lamblia and diarrhoea rates, and to what extent G lamblia modifies the effect of supplementation on nutritional status.

Methods: Data were collected in the context of a randomized placebo-controlled trial with 2x2 factorial design assessing the effects of multi-nutrients (with or without zinc) on morbidity. Children (n=612, aged 6-59 months and height-for-age z-score ≤ 1.5 SD) from a poor rural area were followed for at least
7.4 months after enrolment. Outcomes measures were episodes of diarrhea (any reported, or with ≥ 3 stools in the last 24 hrs) and fever without localizing signs, as detected by clinic based surveillance. *G lamblia* was detected in stool samples by enzyme-linked-immunosorbent assay. Multivariate Cox regression analysis was used to compare disease rates between groups, and to assess interaction effects.

**Results:** Asymptomatic *G lamblia* infection was associated with a substantial protection against diarrhea (HR 0.32; 0.15-0.66) and fever without localizing signs (HR 0.56; 0.36-0.87), but only so among children who did not receive multi-nutrients; no such protection was observed among children who received multi-nutrients (*p*-values for interaction between *Giardia* and multi-nutrients 0.03 for both outcomes, after adjustment for age, HAZ-scores and distance to the dispensary).

**Conclusions/interpretation:** although causality of the *G lamblia*-associated reduction in morbidity cannot be established, the data also show that multi-nutrient supplements neutralize this protection and are thus likely to influence the proliferation of virulence of *G lamblia* or associated intestinal pathogens.
IN VIVO LEISHMANIA PARASITE CLEARANCE

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The pharmacotherapy of leishmaniasis has long been characterized by imprecise monitoring of the treatment response. During treatment clinical observations and blood parameters are used as indicators of treatment response. The parasitological response in visceral leishmaniasis (VL) is usually not followed during treatment because it requires invasive techniques. In the context of clinical trials, parasitological tests are usually performed once after treatment to confirm radical cure; in routine practice repeated parasitological testing is only performed if indicated and possible. For cutaneous leishmaniasis (CL) the treatment response is followed by clinical observation of the lesion, but usually not by repeated sampling. Here we present our first preliminary data of repeated measurement of the Leishmania parasite count over time during treatment of VL and CL.

To investigate if parasite clearance rates can be measured during treatment of leishmaniasis with miltefosine, repeated blood samples from VL patients (4 patients from the AMC, 38 preliminary findings from Kenya and Sudan; all Leishmania donovani/infantum-complex infections) and repeated skin biopsies from CL patients (2 AMC patients -one L. infantum, one L. major infection- with multiple biopsies and 35 military patients with 2 biopsies; all L. major) were collected. The parasite count was measured in blood and skin biopsies by real time RT-PCR.

PCR was positive in blood of all Dutch VL patients, but only of ~80% of African patients. The initial parasite count in Dutch patients ranged from 102 to 103 parasites/ml; in African patients
from nil to 103/ml. During treatment a loglinear decline was observed of approximately 1 log/2 weeks. Skin biopsies of CL patients were always PCR positive with parasite loads as high as 105 parasites per biopsy. During treatment this declined with approximately 1 log/week. The data do not allow distinguishing the clearance of different parasite species. In conclusion, *Leishmania* parasite loads can be quantified in blood and skin biopsies. The results are reproducible and allow for the estimation of parasite clearance rates. This increases the precision and sensitivity to measure treatment response, enables to detect failure at an early stage and improves our pharmacodynamic understanding of leishmaniasis treatment.
Global warming might be expected to favour parasite populations through accelerated development, increasing infection pressure and disease. However, this relationship is compounded by important non-linearities. These may arise from, for example, differing or even contrasting stage-specific rates of development and survival, seasonal fluctuation in temperatures, animal management and human behaviour. Several factors may act in a given system to substantially modify the effect of climate on parasitic disease risk. Thus, accelerated development of the free-living larvae of gastrointestinal nematodes of ruminants in summer can be offset by reduced over winter survival at higher temperatures, leading to unchanged overall levels of infection but shifts in the seasonality of observed disease. In ovine cutaneous myiasis, small changes in animal management such as earlier shearing can largely negate increased infection pressure due to earlier emergence of flies from overwintering pupae. These non-linearities, as well as the lack of control populations, can make the signal of climate change on epidemiological data difficult to detect. In canine angiostrongylosis, for instance, the relative roles of natural time lag in geographical expansion, climate change, and increased awareness cannot be distinguished with any certainty. Epidemiological models that consider climate-driven changes in the epidemiology of animal parasites within their proper social and management context are needed, and must be used critically if predictions of future disease risk are to have foundation.
THE MOST IMPORTANT PARASITES OF FARM ANIMALS IN THE NETHERLANDS

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During the lifespan of the NVP, the veterinary arena of parasite management in the Netherlands has changed a lot. In this short presentation the different decades will be discussed thematically with respect to important parasites or developments that have dominated that specific period. What have we learned from these years and in what way can this be found in current daily veterinary practice?

Starting from Fasciola hepatica, via gastrointestinal nematodes in cattle and sheep to the start of the problem of anthelmintic resistance, the presentation leads us to the introduction of ivermectin followed by the more molecular approach of veterinary parasitology and via the solving of pieces of the Neospora caninum puzzle it ends with some reflections on the paradoxical phenomenon of the growing problem of anthelmintic resistance and the declining number of departments of veterinary parasitology.
Echinococcus multilocularis: The Arrival to Increasing Public Health Risk in the Netherlands

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Alveolar echinococcosis (AE) is one of the most pathogenic parasitic zoonoses in central Europe. Humans are infected when accidentally ingesting the parasite eggs that are shed into the environment by infected foxes. The parasite Echinococcus multilocularis was first detected in the Netherlands in 1996 and it was shown after repeated studies that the parasite subsequently spread in the local population of foxes in the province of Limburg. The likely event of introduction and the subsequent spreading of the parasite in the local fox population raised a concern for an increasing potential of human risk of AE in the coming years. However, the human risk of alveolar echinococcosis was not possible to quantify because no relationship between the amount of the parasite eggs in the environment and the probability of infection in humans was known. Here, we used the spreading of the parasite in the Netherlands as a predictor together with recently published historical records of the epidemiology of alveolar echinococcosis in Switzerland to achieve a relative quantification of the risk. Based on these analyses, the human risk in Limburg was simulated and up to 3 human cases are predicted by 2018. We conclude that the epidemiology of alveolar echinococcosis in the Netherlands might have changed from a period of negligible risk in the past to a period of increasing risk in the forthcoming years.
IMMUNOLOGICAL RESPONSE AGAINST TRICHINELLA SPIRALIS INFECTION IN RATS IS DOSE DEPENDENT

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Trichinella spiralis (T. spiralis) is the only known Trichinella out of 12 recognized species that is transmitted and maintained in both a domestic and sylvatic cycle. The T. spiralis sylvatic cycle involves omnivores like wild boar, carnivores like wolf and fox, and their prey animals like wild rodents. T. spiralis is maintained in pigs as one of the most important representatives of the domestic cycle. In Europe, Asia and Latin America, free ranging pigs of small household farms are the most important risk for public health. Rats and possibly other rodents might play a role in the transmission of Trichinella spiralis from domestic to sylvatic animals and vice versa. Therefore, we studied the dynamics of T. spiralis infection in rats using doses ranging from very low (10 muscle larvae [ML] per rat) to very high (16,000 ML per rat). To augment the dynamics of T. spiralis in infected rats and to evaluate the feasibility of rats surviving high infection doses with T. spiralis, clinical and pathological parameters were also quantified. Serological tools for detecting T. spiralis in rats were developed to quantitatively study the correlation between parasite load and immunological response. Results of the infection experiment showed that a dose dependent antibody response was developed in rats after infection with as low as 10 ML up to a level of 10,000 ML. A clear positive correlation was found between the number of recoverd ML and serum antibody levels. However, the predictive value of measured antibody levels to estimate actual number of intramuscular larvae is limited. Serum antibodies of rats that were infected with 10 or 25 ML, could readily be detected by
use of the *T. spiralis* western blot 2 weeks post infection, which is useful to evaluate sera from animals with low infection levels (or higher infection levels early in the time course of infection) that exhibit antibody titers around cut-off level.
IN VITRO SELECTION FOR IVERMECTIN RESISTANCE WITHIN FOUR HISTORICALLY DIFFERENT ANTHELMINTIC TREATED CYATHOSTOMIN LARVAL POPULATIONS

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Cyathostomins are considered the primary helminth pathogen of horses. Macro cyclic Lactones (ML) are the most commonly used anthelmintics to control the infection pressure on pasture. Resistance against ML would hamper controlling cyathostomins in horses. In this study ivermectin resistant cyathostomin L3 were in vitro selected by a reiterative larval migration inhibition assay (rLMIA). This method is based on the inhibition of migration through 2 consecutive sieves in the presence of ivermectin. L3s before and after selection were differentiated by reverse line blot (RLB), a technique based on the hybridisation of the amplified intergenic spacer sequence with species specific probes from 13 common species. Larvae were obtained from 4 populations: (i) a never treated, free-roaming horse population in the nature reserve Oostvaardersplassen (OVP); (ii) a rarely treated population (CAS); (iii and iv) from regularly ivermectintreated animals (UU and HAI). In the rLMIA the proportion of larvae that migrated increased with each passage, demonstrating a selection for larvae with a decreased susceptibility for ivermectin. In all four populations prior to selection the predominant species were Cyathostomum catinatum, Cylicocyclus nassatus and Cyathostomum pateratum. After in vitro selection the predominant species became C. catinatum, while C. nassatus became
very rare. It is concluded that the rLMIA and RLB can be used to study differences and changes in species and species composition between populations with different anthelmintic exposure histories.
DRUG RESISTANCE IN LEISHMANIA PARASITES

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Visceral leishmaniasis (VL) is a life-threatening, neglected and poverty-related disease caused by Leishmania donovani and L. infantum. Depending on the geographical area and their transmission characteristics, VL is considered to be anthropootic in the Indian subcontinent (L. donovani) and to be zoonotic (L. infantum) in Latin America and the Mediterranean region where domestic dogs serve as the main reservoir. Curing VL is challenging and treatment options are quite limited. For more than seventy years, pentavalent antimonials (SbV) have been the first-line therapy. However, the increasing failure of SbV-based treatment regimens in hyper-endemic areas has become a major concern and isolation of Sb-resistant parasites from unresponsive patients strongly indicates that treatment failure is partly due to drug resistance. In anthropootic VL, selection of resistance is directly related to inappropriate SbV treatment and drug-resistant strains have now become widespread. Hence, man-to-man transmission of drug-resistant lines should be the prime focus of epidemiological monitoring. In zoonotic VL, dogs are currently also treated with antimonials by veterinarians, and since parasitological cure is not obtained, enhanced selection towards drug-resistance becomes easily established with a high probability for direct transmission to man. A better understanding of the factors involved in the selection of drug-resistant mutants is therefore needed with the practical goal to anticipate or overcome the resistance problem and maintain therapeutic efficacy with the currently available drugs.
Although intensive research has been performed in laboratory strains, many unresolved issues have to be faced when considering the definition of ‘resistance’ in the context of the use of antileishmania drugs and characterization of clinical isolates. The first challenge is not just a problem of lack of definition but specifically linked to the unavailability of standardized and validated laboratory assays. Although many in vitro ‘drug screening’ models and procedures have indeed been described during the last decade, standard operating procedures (SOP’s) that will unequivocally define intrinsic drug “susceptibility” as to the in vitro response of a Leishmania strain/isolate to a standard drug are not yet available. Yet another and even more difficult step is the linkage of the in vitro drugsusceptibility profile to clinical outcome in the treated patient since pharmacokinetic, pharmacodynamic and host immune phenomena largely define the response of the pathogen to a standard drug. The second challenge relates to the variation in the response of different strains to the various antileishmania drugs. Difficulties are encountered because drug resistance is frequently defined as a decline in the effectiveness of a drug against a population of parasites previously susceptible to the compound. This definition assumes that the original susceptibility of the population is known, which rarely is the case. The third challenge relates to the absence of definitive knowledge about the mechanisms of action and how this relates to changes in drug susceptibility in clinical isolates from patients who have either responded, relapsed or non-responded to treatment. It is pivotal not just to be able to relate in vitro susceptibility to particular biochemical and/or molecular changes, but also to identify relevant molecular markers that could be used in an epidemiological and clinical setting.

Facing these challenges, a number of focused research initiatives were taken and are briefly summarized here.

**Standardization of the in vitro susceptibility model**

Susceptibility evaluation of patient-derived diagnostic material is hampered by the lack of reliable and reproducible in vitro
procedures and prediction of treatment outcome remains even more speculative. The need for a good definition of drug resistance still exists, particularly because treatment failure for Sb is not necessarily due to drug resistance as the immune system also plays a major role in the elimination of the parasite. An essential step is to establish a standard assay protocol as many models and procedures have been described and are used, however all with small differences such as infection ratio, incubation time, drug exposure range, culture medium, macrophage host cell, etc., making it virtually impossible to compare IC50 values. *In vitro* susceptibility testing in the amastigote-macrophage model is the only valid assay for epidemiological mapping of drug resistance, as molecular markers have not yet been identified neither fully validated. In this work, the standard protocol to assess *in vitro* drug susceptibility of clinical *Leishmania* isolates was therefore adapted. One specific complication is that some field isolates tend to be poorly infective to the macrophage host cell. Considerable variation in metacyclogenesis between different strains and clones is indeed a well-known problem that can be explained by the enhanced spontaneous acidification of the culture medium by rapidly dividing strains compared to slowly growing strains. Spontaneous acidification to trigger the metacyclogenesis process may take a variable number of days dependent on the strain and hence is quite inconvenient when standardization of the assay is projected. To overcome this problem, promastigote cultures can be synchronized and artificially triggered into metacyclogenesis by lowering the pH to 5.4 resulting in a more reproducible infection of macrophages and better performance of the *in vitro* assay.

Yet another even more difficult step is to link the *in vitro* drug-susceptibility profile to the clinical outcome because pharmacokinetic, pharmacodynamic and host immune phenomena considerably mediate the response of the pathogen to a standard drug. In order to consider a pathogen population as resistant, a clear definition of the drug concentration that separates susceptible from non-susceptible populations is needed. The
activity index (AI) can be considered as a practical tool for the definition of Sb-resistant strains, as it expresses the *in vitro* susceptibility of the tested strain in comparison to a drug-sensitive reference strain. The cut-off concentration for ‘resistance’ must be based upon the *in vitro* susceptibility of a large number of clinical or environmental isolates. Susceptible reference strains include *L. donovani*: MHM/ET/67/L82 and *L. infantum* MHOM/MA/67/ITMAP263, for which IC50 values have been well established. The AI has proven to be a robust tool for comparison of results between different series of experiments and even between laboratories. Based on available data from different laboratories, the cut-off value for Sb-resistance was set at AI >4. When AI values for SbV or SbIII are evaluated separately, *in vitro* results may not correlate with the *in vivo* treatment outcome. This can be explained by the presence of Sb resistant strains in a large number of cured patients and the frequent presence of SbIII susceptible strains in non-responders or relapse cases. However, combining AI-values for both SbV and SbIII improves the association with treatment outcome, linking ‘S/S’ to cure and ‘R/R’ to nonresponders or relapse cases (p = 0.023). The ‘R/S’ phenotype could be considered as an intermediate profile with increased risk to evolve to R/R, but there is yet no clinical evidence for this hypothesis. Clinical outcome in patients infected with a ‘R/S’ strain was variable, and possibly relates to differences in immune competence. Further research is needed to characterize this ‘R/S’ profile and how it evolves under continued drug pressure and affects clinical outcome. To answer the question whether ‘R/S’ isolates would evolve more rapidly to ‘R/R’ profiles, a large number of pre- and post-treatment isolates from Sb-treated patients should be studied, but with keeping in mind that also host factors do influence treatment outcome and are far more difficult to consider in an *in vitro* laboratory model.

**Monitoring drug resistance**

The *Leishmania* research community needs to set basic requirements for surveillance and monitoring of drug susceptibility
in disease control and provide relevant information as to
guide treatment strategies. With the improved standardized
susceptibility assay, several pilot field studies have been performed.
In a first study, indications about the dynamics of Sb-resistance
were obtained from a Brazilian study in dogs naturally infected with
*L. infantum* and treated with Sb. The evolution towards resistance
appeared to be stepwise from S/S to R/S in a first phase and
to R/R in a later phase. This conclusion should be interpreted with
some caution since it is based on a relatively small number of
samples. However, highly relevant indications were obtained:
selection of Sb-resistant parasites may occur rapidly during
treatment and these resistant mutants may be transmitted to man
in a zoonotic transmission cycle resulting in primary
unresponsiveness.

In a second study, *L. infantum* strains were collected from HIV-co-
infected patients in South-America and France. It appears that SbV
resistance is quite common with the Sb R/S profile being most
widely dispersed in both endemic areas. In the Brazilian focus, this
profile was quite common and equally distributed among HIV+ and
HIV- patients. This phenomenon is not expected in the context of
zoonotic *L. infantum* since treatment of dogs
is officially prohibited in Brazil. Dissemination of primary SbV-
resistant isolates is a phenomenon generally expected in
anthropogenic transmission where the parasite is likely
to be under a stronger drug pressure than in zoonotic
leishmaniasis. Consequently, two possibilities could cause the large
spread of R/S strains in the Brazilian focus: either a more
widespread veterinary practice of canine leishmaniasis treatment
with ensuing selection and dissemination of resistant parasites, or a
greater impact of anthropogenic transmission than initially assumed.
Close monitoring should be warranted to study the emergence and
evolution of the R/S parasites in this endemic area because SbV is
still the first-line treatment.

The situation in the Mediterranean region is quite different since
liposomal amphotericin B is used as first-line therapy. the *in vitro*
Sb-susceptibility on isolates from HIV co-infected patients was
determined and Sb-resistance (R/R or R/S) proved to be very common, even though the patients never came into contact with SbV. This phenomenon can only be explained by the fact that treatment of canine leishmaniasis with Sb is routine veterinary practice, whereby dogs function as a reservoir for infection of man. Although a large spread of R/S parasites was observed for both endemic areas, the specific transmission patterns remain largely unknown. Primary Sb-unresponsiveness would be most problematic in the Brazilian focus where Sb is still used as the first-line treatment.

Continued monitoring of drug resistance remains therefore a priority and actual treatment options in man and dog need to be tailored to conserve all treatment options for VL in the near future.

**Selection or experimental induction of drug resistance**

A better understanding of the factors involved in the selection of drug-resistant mutants would serve the ultimate goal to circumvent or overcome the resistance problem. Studies of resistance mechanisms cannot reverse the tide for Sb but they can help handle the current situation by developing tools to either recognize resistance early during infection or to monitor drug resistance in the field and develop strategies to prevent further dissemination of resistant parasites. However, due to the multifactorial nature of Sbresistance and its complexity, many aspects still remain obscure. Paromomycin (PMM) is currently under investigation for its potential to replace SbV as a first-line drug. Once routinely used in the field, the risk of development of resistance is real, endorsing the short-term need to study PMM resistance mechanisms. At present, very little is known on PMM resistance, partly also because resistant clinical isolates are not yet available. To study drug resistance mechanisms proactively, drug-resistant lines need to be induced in the laboratory. A novel approach was implemented adopting the principle of selection of drug-resistant parasites at the intracellular amastigote level, mimicking the natural course as close as possible. The basic principle of the method was to maintain the highest
possible drug pressure during the alternate cycles of promastigotes used to infect macrophages and intracellular amastigotes. Amastigotes surviving the highest drug concentration were allowed to transform back to promastigotes to allow expansion of the population, either adopting continued drug pressure (at half the IC50) or not. These next generation promastigotes were then used for infection of macrophages under higher drug pressure. These selection cycles are repeated until the maximum level of resistance was reached.

A resistance induction experiment was performed for PMM on a clone with Sb-resistant (R/R) background and with drug selection being applied on intracellular amastigotes. PMMresistant parasites could already be selected after two cycles tolerating up to 4.5x higher concentrations compared to the parent strain. The induced strain was subsequently cloned and proved to be polyclonal: some clones were still fully susceptible to PMM while others were highly resistant with tolerance levels up to 10x compared to the original strain.

Consistent with previous publications, cross-resistance with other antileishmania drugs was not present: the in vitro susceptibility of the original clone for MIL (susceptible) and Sb (R/R profile) was preserved in the induced clones, even after passage in hamsters. If such selection would actually occur in the field is unknown, but its implications would be disastrous. These observations also endorse the need to adopt stringent treatment policies to ensure long term efficacy of PMM. These resistant mutants now also provide a good opportunity to explore PMM resistance mechanisms in great detail. This also illustrates the selection strength of the model and underlines the fact that Leishmania spp. can fairly easily adapt to drug pressure.
African Animal Trypanosomiasis causes the death of 3 million head of cattle each year. The annual economic losses as a result of the disease are estimated to be 4.5 billion US dollars. Trypanosomes are transmitted by tsetse flies and can infect a wide range of hosts from wildlife to domestic animals. This study is dealing with Trypanosoma congolense, which is one of the very prevalent parasites affecting livestock of poor African rural communities, decreasing the milk and meat production but also reducing the fitness of cattle that is used as draught power. Infected animals can only be treated by three compounds, i.e., diminazene aceturate, isometamidium chloride (ISM) and ethidium bromide. These three products have been in use for more than a half century and it is thus not surprising to observe treatment failures. In some areas, the trypanosomes circulating have developed resistance to the three drugs leaving the farmers with no further options. As pharmaceutical companies are not keen on investing efforts and
money in the development of new veterinary drugs for this low-budget market, our idea was to render an old ineffective drug effective again by combining it with existing potentiating compounds that are available and affordable for the livestock keeper. We may thus have found a breakthrough in the treatment of resistant trypanosomal infections, through the combination of the trypanocide ISM with two affordable veterinary antibiotics. In a first experiment, groups of mice were inoculated with *Trypanosoma congolense* strains resistant to ISM and either left untreated or treated with (i) tetracycline, (ii) ISM or (iii) the combination of the antibiotic and the trypanocide. Survival analysis showed that there was a significant effect of treatment and resistance to treatment on the survival time. The groups treated with ISM (with or without antibiotic) survived significantly longer than the groups that were not treated with ISM (P<0.01). The group treated with the combination trypanocide/antibiotic survived significantly longer than the group treated with ISM (P<0.01). In a second experiment, groups of cattle were inoculated with the same resistant trypanosome strain and treated with (i) ISM, (ii) ISM associated with oxytetracycline or (iii) ISM associated with enrofloxacine. All animals treated with ISM became parasitaemic. In the groups treated with ISM-oxytetracycline and ISM-enrofloxacine, 50% of the animals were cured. Animals from the groups treated with a combination trypanocide/antibiotic presented a significantly longer prepatent period than animals treated with ISM (p<0.001). The impact of the disease on the haematocrit was low in all ISM treated groups. Yet, it was lower in the groups treated with the combination trypanocide/antibiotic (p<0.01).

After optimization of the administration protocol, this new therapeutic combination could constitute a promising treatment for livestock infected with drug resistant *T. congolense*. 
NEW DEVELOPMENTS IN MOLECULAR DETECTION OF GUTFLORA AND THE PRESENCE OF PARASITES

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Currently, many new molecular approaches are being developed to detect parasites in clinical specimens. These tests replace more and more classic parasitology while introducing new possibilities for detection of many different species. However this results in many different PCR’s that should be performed while not resolving a clinical role between putative different subtypes or variants of parasites. With regard to Blastocystis spp. there are many contradictions in literature concerning the pathogenicity of different subtypes. We introduce a new approach to be able to detect parasites while in the meantime discriminate different subtypes directly from clinical samples.

The method is based on the bacterial IS-pro method. This method uses 16S-23S length polymorphism to detect and profile all bacterial species in the gut microflora. The same principle is used for detecting all parasites present and putative subtypes. For parasites the method detects length differences between the 18S-28S region. The first results and putative possibilities of this approach will be presented as an example of a new single PCR approach with subtype discrimination of parasites directly from clinical samples.
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