First Scientific Meeting of the joint Belgian Society for Parasitology and Protistology

“Unity in diversity”

Thursday 7 November 2013

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Antwerp

www.parasitology.be

1st MEETING OF THE JOINT BELGIAN SOCIETY OF PARASITOLOGY AND PROTISTOLOGY

In this inaugural meeting of the joint Belgian Society of Parasitology and Protistology, abbreviated BSPP, the theme "Unity in Diversity" is very appropriate to illustrate the spirit and scope of the two mother societies condensed into the joint BSPP society.

Historical perspective

The Belgian Society for Parasitology and the Belgian Society of Protozoology, sharing the same acronym, have been founded in respectively 1962 and 1966. The Parasitology society was established under impulse of Prof. Jean-Baptiste Jadin and Prof. Alex Fain, two parasitologists at the Institute of Tropical Medicine of Antwerp. The Protozoology society was founded as subsidiary of the International Society of Protistologists (ISP) under the impulse of Prof. Percy Garnham (LSTM) and Prof. Jean-Baptiste Jadin. The Parasitology society counted about 80 members by 2012 at which time the Protozoology society had about 30 subscribed members. Both societies have been exploring the possibility of a merger for quite some time. The first formal attempt was made in 1991, yet the minds were not ready to shape a joint Society that captured the spirit and scopes of both Societies. Since 2002, the two boards have reiterated this idea, and agreed to propose the merger to their members at the respective 2012 statuary meetings where the fusion was approved.

Joint goals set

The activities of the joint BSPP Society have been expanded and are oriented at the study of parasites (arthropods, helminthes and protozoa) and their vectors as well as free living protists. This unrestrictedly includes the study of the organism's morphology, taxonomy, biology, biochemistry, physiology, ecology and pathological and immunological interactions with their vertebrate and invertebrate hosts as well as the epidemiology and control of infections caused by parasites. The main incentive is to promote the cooperation at national and international level between those who have interest in these disciplines and to support young scientists in the development of their career.

The annual scientific meeting

The BSPP will honor the tradition to hold one full day annual scientific meeting with lectures by invited speakers and presentations, mainly by young researchers. There has also been a tradition of organizing joint meetings with other societies such as the British and Dutch Societies of Parasitology, which is important for the ambition of BSPP to be active on the international scene. At the day of the scientific meeting, also a statutory meeting is held to discuss BSPP issues and to vote proposed changes in the statutes.

Supporting young scientists

To encourage the young scientists, BSPP will continue to create awards with the support of sponsors as it was the tradition for many years in the two mother societies. This year, a travel grant has already been awarded to a protozoologist (Carole Haynes) for attending the 17th Annual Woods Hole Immunoparasitology Meeting. There is also the Zoetis travel grant, previously the Pfizer Animal Health travel grant that has been created to support young PhD researchers to attend an international conference. This year Lynn Meurs is awarded the Zoetis grant for attending the 62nd Annual Meeting of the American Society of Tropical Medicine and Hygiene in Washington DC. As it has been a tradition since 2005, there is also an award for the best presentation at this BSPP meeting which has been sponsored by Janssen Animal Health from 2006 and by Elanco from 2012.

Statutes of the new joint society

At this meeting members are invited to discuss and approve the statutes of the society.

With the joining of forces of parasitologists and protistologists, the scientific program is covering a new and broad range of research fields and we hope you will enjoy it.

The BSPP committee

PROGRAMME

09:30	Registration and coffee	
Session 1 – Chair: Louis Beyens		
10:00	BSPP Presidents	Welcome address
10:15	Keynote: PROF. JOANNE P. WEBSTER (IMPERIAL COLLEGE, LONDON, UK)	EPIDEMIOLOGY, EVOLUTION AND CONTROL OF SCHISTOSOMIASIS IN A CHANGING WORLD
10:55	Coffee break	
11:15	A. Kassem	MONOALLELIC VARIANT SURFACE GLYCOPROTEIN EXPRESSION IN *TRYPANOSOMA BRUCEI* IS CONTROLLED DOWNSTREAM FROM TRANSCRIPTION INITIATION.
11:30	G. Boulet	EXPERIMENTAL SELECTION OF PAROMOMYCIN AND MILTEFOSIN RESISTANCE IN INTRACELLULAR AMASTIGOTES OF *LEISHMANIA DONOVANI* AND *L. INFANTUM*
11:45	J. Cnops	*TRYPANOSOMA BRUCEI BRUCEI* INDUCED ACUTE INFLAMMATION IS PRINCIPALLY MEDIATED BY IFNG
12:00	A. Fortin	DRUG DELIVERY BY TATTOOING TO TREAT CUTANEOUS LEISHMANIASIS: A PROOF OF CONCEPT
12:15	E. Obishakin	CHRONIC *TRYPANOSOMA CONGOLENSE* INFECTIONS IN MICE CAUSE A SUSTAINED DISRUPTION OF THE B CELL HOMEOSTASIS IN THE BONE MARROW AND SPLEEN
12:30	Lunch	
13:10	BSPP general meeting	
Session 2 – Chair: Jan Van Den Abbeele		
14:00	Keynote: PROF. DR. THORSTEN STOECK (UNIVERSITY OF KAISERSLAUTERN, GERMANY)	LIVING IN EXTREMES – PROTISTS IN HIGH-SALT ENVIRONMENTS
14:40	E. Lambrecht	*YERSINIA ENTEROCOLITICA* BEHAVIOR IN THE PRESENCE OF THE BACTERIVOROUS ACANTHAMOEBA CASTELLANII.
14:55	J. Baré	INTERACTION OF *ASPERGILLUS FUMIGATUS* CONIDIA WITH *ACANTHAMOEBA CASTELLANII* PARALLELS MACROPHAGE-FUNGUS INTERACTIONS
15:10	F. Kauffmann	*LEISHMANIA DONOVANI* B CELL DYSFUNCTION IS NOT MEDIATED THROUGH B CELL DEPLETION
15:25	W. Vyverman	LIFE CYCLE REGULATION AND SEX DETERMINATION IN PENNATE DIATOMS (STRAMENOPILA)
15:40	K. Sabbe	FUNCTIONAL ECOLOGY OF MARINE INTERTIDAL DIATOMS: LINKING PHOTOPHYSIOLOGY TO COMMUNITY ECOLOGY
15:55	Coffee break	

Session 3 – Chair: Louis Maes		
16:15	G.H. Grit	IMMUNITY AGAINST *GIARDIA DUODENALIS* INFECTION IN CATTLE
16:30	R. Keshav	RELAPSE AFTER TREATMENT WITH MILTEFOSINE FOR VISCERAL LEISHMANIASIS IS ASSOCIATED WITH INCREASED INFECTIVITY OF THE INFECTING *LEISHMANIA DONOVANI* STRAIN.
16:45	B.T. Dung	TRANSMISSION ASPECTS OF FASCIOLOSIS IN VIETNAM
17:00	W. Tack	THE IMPACT OF FOREST CONVERSION ON TICK POPULATIONS
17:15	Elanco & Zoetis awards	
17:30	Reception	
19:00	Dinner	

INVITED SPEAKERS

EPIDEMIOLOGY, EVOLUTION AND CONTROL OF SCHISTOSOMIASIS IN A CHANGING WORLD

Joanne P. Webster
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Environmental change, through natural and/or anthropogenic factors, can modulate infection prevalence, intensity and disease severity. For schistosomes, the causative agents of the water-borne disease schistosomiasis, recent changes in selective pressures following, for instance, increased mass drug administration programmes, combined with new dam constructions/irrigation systems and/or altered agricultural practices, may all impact the availability of suitable definitive and intermediate hosts for such parasites, and hence potential for both intra- and inter-specific interactions within such hosts. Furthermore, when humans and their livestock come into closer water contact, novel zoonotic hybrid schistosome species may be predicted to evolve and establish as a consequence, with subsequent change in parasite life history traits, transmission potential and virulence. Here, using empirical examples from carefully controlled laboratory experimentally induced selective pressures, from Asia (where schistosomes have been exposed to longer term selective pressures) and Africa (where schistosomes are currently being exposed to recent novel selective pressures). The dynamic nature of these parasites and their potential for evolutionary change under natural conditions is demonstrated. Such studies serve to illustrate the importance of fully understanding the patterns of schistosome distribution and transmission across changing environment if we are to ever hope to achieve the ultimate aim of controlling this disease of profound medical and veterinary importance.

LIVING IN EXTREMES – PROTISTS IN HIGH-SALT ENVIRONMENTS

Thorsten Stoeck
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About two decades ago, geologists discovered a new habitat in the Eastern Mediterranean Sea. Subsequent research revealed that these habitats are lakes at the bottom of the sea, located in a depth of ca. 3500 m below sea level. These lakes originated from the Messinian salinity crisis (late Miocene, ca. 5 Mio. years ago) and are characterized by saturated salt concentrations, methane, hydrogen sulphide and anoxia. The combination of these factors makes these deep hypersaline anoxic basins (DHABs) to one of the most hostile and extreme places on our planet. Until recently, these conditions were thought to be anathema to life. However, initial biological research project demonstrated that against all odds, these DHABs are teeming with a high diversity of active prokaroytes. Motivated by these data, we in 2008 set out to start our research on the diversity and structures of protistan communities in these unique habitats. During several cruises we applied an array of microscopy-based as well as molecular approaches to unlock the hidden secrets of this world. We not only revealed new bizarre life in these basins, but also used their model character to study basic principals of ecology, including the role of environmental filtering and species sorting in structuring of protistan communities. My presentation will be a diary of this journey into the depths of the Eastern Mediterranean Sea that we have started in 2008.

ORAL PRESENTATIONS

MONOALLELIC VARIANT SURFACE GLYCOPROTEIN EXPRESSION IN *TRYPANOSOMA BRUCEI* IS CONTROLLED DOWNSTREAM FROM TRANSCRIPTION INITIATION.

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African trypanosomes survive the immune defence of their hosts by regularly changing their antigenic coat made of Variant Surface Glycoprotein (VSG). The *Trypanosoma brucei* genome contains more than 1,000 VSG genes. To be expressed, a given VSG gene has to be located in one of fifteen telomeric regions termed VSG expression sites (ESs), which each contain a polycistronic transcription unit that includes ES-associated genes (ESAGs). Only one ES is fully active at a time, so that only one VSG gene is transcribed per cell. Although this monoallelic expression is controlled at the transcriptional level, the precise molecular mechanism is still not understood. Here we report that in single cells transcription is initiated on several ESs simultaneously, indicating that the monoallelic control occurs downstream from transcription initiation.

EXPERIMENTAL SELECTION OF PAROMOMYCIN AND MILTEFOSIN RESISTANCE IN INTRACELLULAR AMASTIGOTES OF *LEISHMANIA DONOVANI* AND *L. INFANTUM*

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Objectives: Since antimony treatment failure rates have increased during the last decade, management of visceral leishmaniasis becomes more dependent on alternative therapeutics such as paromomycin (PMM) and miltefosine (MIL). Although widespread resistance has not yet been demonstrated in clinical isolates, both drugs run the risk of selecting for resistance once more routinely used in the field. Hence, unraveling the dynamics and mechanisms of PMM- and MIL-resistance in *Leishmania* holds a key role in managing resistance development and spread in the field.
Methods: Resistance against PMM and MIL was experimentally selected in *L. donovani* and *L. infantum* strains by exposing intracellular amastigotes to increasing drug concentrations in successive selection cycles. The susceptibility endpoint (IC_{50}) was based on intracellular amastigote counting upon Giemsa staining and on promastigote back-transformation. These assays were also applied to field isolates of MIL-relapse patients to assess their diagnostic validity for resistance.
Results: PMM-resistance could readily be selected in amastigotes of all strains, whereas full PMM-susceptibility was maintained at the promastigote stage. Intracellular amastigote counting upon Giemsa staining did not show a decreased MIL-susceptibility after successive MIL-resistance selection cycles. However, promastigote back-transformation still occurred at higher MIL-concentrations suggesting the presence of 'residual' MIL-resistant intracellular stages. The recovered promastigotes proved to be MIL-susceptible. The same phenomenon was observed in a set of clinical isolates from MIL-relapse patients.
Conclusions: The results of the present study endorse the need to use the intracellular amastigote assay for the diagnosis and monitoring of PMM-resistance in patient isolates. The promastigote back-transformation assay could be highly relevant for the detection of MIL-resistance emergence.

TRYPANOSOMA BRUCEI BRUCEI INDUCED ACUTE INFLAMMATION IS PRINCIPALLY MEDIATED BY IFNG

Jennifer Cnops, Carl De Trez, Stefan Magez

In murine *Trypanosoma brucei brucei* infection the onset of inflammation occurs rapidly during the first days post infection, as witnessed by hepatosplenomegaly and a burst of pro-inflammatory cytokine levels in the serum. IFNg is one of the major cytokines driving this acute inflammatory reaction. Using IFNg reporter mice we show that the liver is the main organ responsible for IFNg production. Hepatic NK cells react within 24 hours and are the main producers of IFNg during the first 3 to 5 days post infection (pi). Subsequently CD8 T cells are activated and take over IFNg secretion. After about 8 days pi, CD4 T cells seem to become the main source of IFNg. IFNg is partly responsible for 2 major pathologic events occurring during trypanosomiasis infection. Firstly, IFNg- and IFNgR-deficient mice display diminished acute anemia compared to WT mice, in which red blood cell serum levels drop to 50% at day 6 pi. While CD4-/- and CD1d-/- mice exhibit a similar WT phenotype, CD8-/- mice exhibit a phenotype which is similar to IFNg-/- and IFNgR-/- mice, implicating CD8 T cells in IFNg production. Secondly, IFNg seems to play a role in the characteristic B cell depletion occurring during murine trypanosomiasis infection, as IFNg-/- and IFNgR-/- mice exhibit less depletion and less apoptosis in splenic B cell subsets. Therefore we hypothesize IFNg driven inflammation to be a cause of B cell depletion during murine trypanosomiasis infection.

DRUG DELIVERY BY TATTOOING TO TREAT CUTANEOUS LEISHMANIASIS: A PROOF OF CONCEPT

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Leishmaniasis is a vector-borne disease caused by obligate intra-macrophage protozoa of the *Leishmania* species. Leishmaniasis can cause different clinical syndromes, including cutaneous leishmaniasis (CL), in which the patient presents with one or several ulcer(s) or nodule(s) in the skin resulting from the infection of macrophages located in the dermis. Although there is a huge clinical need, no good treatment is available for CL at the moment. This project investigates the hypothesis that a tattoo device can target intradermal drug delivery against CL parasites.
The selected drug is oleylphosphocholine, an alkylphosphocholine in the same family as miltefosine which can be formulated as nanoliposomes that can be delivered by tattooing. *In vitro* efficacy of the formulated drug was first established in cultured macrophages. Subsequently, mice infected with *L. major* or *L. mexicana* and presenting skin lesions were treated using a 10-day regimen. *In vivo* efficacy of the tattoo-mediated drug delivery was evaluated at the clinical and parasitological levels and compared to other more classical modes of administration.
Cultured *Leishmania*-infected macrophages can engulf drug-containing nanoliposomes resulting in a direct dose-dependent killing of intracellular parasites. On infected mice with cutaneous lesions, a 10-day tattoo-mediated treatment resulted in rapid clinical recovery with complete regression of skin lesions. Parasite counts and histopathological examination confirmed efficacy of the treatment. No damage was observed to the skin architecture beyond those normally seen from the tattooing procedure.

This study establishes proof-of-concept that localized tattoo-mediated delivery of an antileishmanial drug is effective in treating CL. The low amount of drug required and the high efficacy of this treatment method may have a positive impact on CL patient management. Future studies will

measure drug levels in the skin layers over time following tattoo-mediated therapy in view of transposing the results to clinical studies.

CHRONIC *TRYPANOSOMA CONGOLENSE* INFECTIONS IN MICE CAUSE A SUSTAINED DISRUPTION OF THE B CELL HOMEOSTASIS IN THE BONE MARROW AND SPLEEN

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Trypanosoma congolense is one of the main species responsible for Animal African Trypanosomosis (AAT). As preventive vaccination strategies for AAT have been unsuceesful so far, investigating the mechanisms underlying vaccine failure has to be prioritized. In *T. brucei* and *T. vivax* infections, recent studies revealed a rapid onset of destruction of the host B cell compartment, resulting in the loss of memory recall capacity. To assess such effect in experimental *T. congolense* trypanosomosis, we performed infections with both the cloned Tc13 parasite, which is considered as a standard model system for *T. congolense* rodent infections, and the non-cloned TRT55 field isolate. These infections differ in their virulence level in the C57BL/6 mouse model for trypanosomosis. We show that early on, an irreversible depletion occurs of all developmental B cells stages. Subsequently, in the spleen, a detrimental decrease in immature B cells takes place, followed by a significant and permanent depletion of Marginal Zone B cells and Follicular B cells. The severity of these events later on in infection correlated with the virulence level of the parasite stock. In line with this, it was observed that later-stage infection-induced IgGs were largely non-specific, in particular in the more virulent TRT55 infection model.

YERSINIA ENTEROCOLITICA BEHAVIOR IN THE PRESENCE OF THE BACTERIVOROUS *ACANTHAMOEBA CASTELLANII*.

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Free-living protozoa are ubiquitous in natural aquatic and terrestrial environments and present in diverse anthropogenic and food related habitats (e.g. meat cutting plants, refrigerators). Free-living protozoa are bacterial predators, but some bacteria are able to evade protozoan uptake and/or digestion, turning the protozoon into a reservoir, shelter, vector or virulence training ground. *Yersinia enterocolitica* is the third most reported foodborne pathogen in Europe and is associated with the consumption of raw or insufficiently heated pork.
In vitro cocultivation assays were set up to test if *Y. enterocolitica* is resistant to predation by the protozoon *Acanthamoeba castellanii*. Furthermore, it was assessed if environmental factors and bacteria specific characteristics influence this interaction. Therefore, four *Y. enterocolitica* strains with different virulence properties (absence or presence of the *Yersinia* Virulence plasmid pYV) and different serotypes (4/O:3 and 2/O:9) were cocultivated with *A. castellanii*.
The four *Y. enterocolitica* strains resisted predation by *A. castellanii* for at least 14 days, irrespective of medium (nutrient-rich/poor) and temperature (7, 25 and 37°C) used. Moreover, association with *A. castellanii* significantly enhanced the survival of the *Yersinia* strains under nutrient rich conditions at 25°C. Factors excreted by one *Y. enterocolitica* strain showed a temperature-dependent permeabilizing effect on the protozoa. Long-term intraprotozoan survival of *Y. enterocolitica* was dependent on nutrient availability and temperature, with up to 2.8 log cfu/ml bacteria surviving intracellular at 7°C for at least four days in nutrient-rich medium. Transmission electron microscopy revealed that intra-amoebal yersiniae were located in the amoebal cytosol.
As *Yersinia* and *Acanthamoeba* share similar ecological niches, this interaction suggests a role of free-living protozoa in the ecology and epidemiology of *Y. enterocolitica*. Further research is needed to

unravel the advantages of this interaction for *Y. enterocolitica* and the underlying cell biological mechanisms involved.

INTERACTION OF *ASPERGILLUS FUMIGATUS* CONIDIA WITH *ACANTHAMOEBA CASTELLANII* PARALLELS MACROPHAGE-FUNGUS INTERACTIONS

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Aspergillus fumigatus and free-living amoebae are inhabitants of common natural environments. Free-living amoebae, including members of the genus *Acanthamoeba*, are microbial predators. Nevertheless, various microorganisms resist amoebal grazing and/or digestion, which promotes their survival in the environment. Free-living amoebae can also act as biological training grounds, enhancing the virulence of human pathogens against their host cells.
We hypothesize that *A. fumigatus* acquired its virulence against mammalian and avian hosts by using free-living amoebae as training ground. Therefore, interactions of *A. fumigatus* conidia with *A. castellanii* trophozoites are investigated.
Cocultivation assays with *A. fumigatus* and *A. castellanii* were performed to evaluate their interaction. Besides enumeration, viability of *A. castellanii* was assessed by trypan blue exclusion assays. Epifluorescence microscopy was performed to evaluate the phagocytic capacity of *A. castellanii*, while light and transmission electron microscopy were used to observe the trafficking of *A. fumigatus* inside *A. castellanii.*
Approximately 25 % of the amoebae ingested *A. fumigatus* conidia after 1 h of contact. During intra-amoebal passage, part of the ingested conidia were able to escape the food vacuole and to germinate inside the cytoplasm of *A. castellanii*. Fungal release into the extra-protozoan environment by exocytosis of conidia or by germination was observed. These processes resulted in structural changes in *A. castellanii*, leading to amoebal permeabilization without cell lysis.
In conclusion, *A. castellanii* internalizes *A. fumigatus* conidia, resulting in fungal intracellular germination and subsequent amoebal death. As such, this interaction highly resembles that of *A. fumigatus* with mammalian and avian macrophages (Van Wayenberghe L, 2012, Vet Res 43:32-36). This suggests that *A. fumigatus* virulence mechanisms to evade macrophage killing may be acquired by co-evolutionary interactions among *A. fumigatus* and environmental amoebae.

LEISHMANIA DONOVANI B CELL DYSFUNCTION IS NOT MEDIATED THROUGH B CELL DEPLETION

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Visceral leishmaniasis (VL) is a neglected zoonotic disease that is caused by the bite of phlebotomine sandflies. Infected individuals exhibit hepatosplenomegaly and polyclonal hypergammaglobulinemia. Infection in the liver is controlled, whereas the spleen displays uncontrolled parasite growth, massive micro-architecture remodelling, leading to severe immunosuppression. B cells have been demonstrated to play a negative role during VL. B cell depletion was shown to enhance resistance to *Leishmania donovani* infections and marginal zone B cells were proven to suppress Ag-specific CD8 and CD4 T cell responses during the early stages of VL. However, the mechanisms underlying B cell dysfunction during VL infections are still poorly understood. Considering the phylogenetic

acquaintance of *Leishmania spp.* to *Trypanosoma spp.*, both of which belong to the Trypanosomatidae, we hypothesized that Leishmania parasites modulate the host immune system by exhausting the B cell compartment, as this occurs during *T. brucei* infections. In this study, we investigated the impact of *L. donovani* infection on the distribution of various B cell subsets, in the liver and spleen of C57BL/6 mice and BALB/c mice. The percentages of B cell subsets, including immature, transitional and mature B cells did not differ significantly between infected and control mice over the course of infection. No significant differences were found between infected and control mice at the level of T cell subsets, NK cells, myeloid cells or granulocytes either. Although not one population seemingly changed during the course of infection in both C57BL/6 and BALB/c mice, the total cell amounts were higher in infected mice compared to control mice, indicating a certain level of inflammation. Also, signs of infection, such as mild hepatosplenomegaly and the presence of parasites in liver and spleen were observed. Therefore, our data suggest that depletion of the B cell compartment is not a hallmark of experimental *L. donovani* infections.

LIFE CYCLE REGULATION AND SEX DETERMINATION IN PENNATE DIATOMS (STRAMENOPILA)

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Sex is an obligatory and essential process in the life cycle and hence the population and bloom dynamics of most diatoms. To date however, the molecular-genetic basis of sex determination in the diatoms is unknown. The pennate marine diatom *Seminavis robusta* is emerging as a model system to study the molecular regulation of sexual reproduction in diatoms. In this heterothallic species (with two mating types, MT^+ and MT^-), sexual crosses can easily be experimentally manipulated and synchronized, allowing full experimental control of the sexual process. Bioassay-guided fractionation of extracellular metabolites resulted in the identification of a conditioning factor and an attraction pheromone. The former molecule induces a delay in cell-cycle progression and up-regulation of the production of the attraction pheromone diprolin. Mating type specific linkage maps revealed a single locus sex determination system, with MT^+ as the heterogametic sex. Bulked segregant analysis in combination with AFLP and NGS allowed fine-mapping the sex locus, which encodes a helicase. We then used a comparative genomics approach to identify the sex locus in sequenced diatom genomes, and to study the evolution of the mating type locus within the diatoms.

FUNCTIONAL ECOLOGY OF MARINE INTERTIDAL DIATOMS: LINKING PHOTOPHYSIOLOGY TO COMMUNITY ECOLOGY

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Despite sharp and dynamic gradients in light availability, physical disturbance and biogeochemistry, intertidal sediments belong to the most productive ecosystems on Earth. In temperate regions, they are typically inhabited by dense benthic diatom communities, which are highly diverse. Ecological studies have revealed the presence of a number of distinct growth forms: (1) the epipelon comprises large motile diatoms which move freely in between sediment particles; (2) the epipsammon, which groups smaller diatoms which live attached to (or otherwise closely associated with) individual sand grains; and (3) the tychoplankton, an ill-defined and enigmatic consortium of largely non-motile diatoms which presumably have an amphibious life style (both sediment and water column). All growth forms show distinct distribution patterns in time and space, suggesting pronounced (micro)niche differentiation. We hypothesized that this niche differentiation is related to functional features of these growth forms. Diatoms have evolved many physiological processes in order to acclimate to the changing light climate and especially to resist stressful light conditions. Non-photochemical quenching of chlorophyll fluorescence (NPQ) and the associated xanthophyll conversion (XC) from diadinoxanthin (DD) to diatoxanthin (DT) are believed to be one of the most important short-term photoprotective processes. We compared NPQ and XC performance and kinetics of different epipelic, epipsammic and tychoplanktonic species. Our results suggest that differences in growth form and behavior (motility) have driven functional adaptations (i.c. photoprotective capacity and strategies) of benthic diatoms to their respective microhabitats, and that these functional differences contribute to the structuring and dynamics of intertidal benthic diatom communities.

IMMUNITY AGAINST *GIARDIA DUODENALIS* INFECTION IN CATTLE.

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Giardia duodenalis causes diarrhoea in humans and a wide range of mammals, including cattle. The infection often has a chronic character. Infected calves excrete cysts for several months, suggesting that the development of protective immunity is suppressed by *Giardia*.

Six calves were infected with *G. duodenalis* assemblage A and E and housed in an environment that allowed reinfection. Cyst excretion was monitored two times per week until decreasing cyst counts indicated the development of protective immunity. The kinetics of the circulating memory cells and serum antibodies were followed up until the animals were considered immune. Cyst excretion started 2 weeks post infection and remained high until week 14. Low cyst counts from week 15 p.i. onwards indicated that the calves had developed immunity.

Serum IgG1 and IgA levels against *G. duodenalis* assemblage A and E increased from week 11 of the infection and high levels of parasite-specific IgA were present in the intestinal mucus at necropsy (week 20). From early in the infection, all trophozoites stained positive in an IFA assay with serum antibodies, indicating that a broad repertoire of antibodies was produced against all variant-specific surface proteins.

From week 5 p.i. a significant proliferation of $CD4^+$ and $CD8^+$ αβ T-cells was observed after *in vitro* stimulation with *G. duodenalis* antigen. This proliferation was reduced after plastic adhesion of the PBMC, suggesting a role for antigen-presenting cells. Characterisation of the proliferating $CD4^+$ T-cells using real time qPCR showed high transcription levels of Foxp3 and IL-17. Further research is necessary to tell whether IL-17 is produced by FoxP3 cell or that two different cell populations are present, FoxP3 regulatory T-cells and Th17 cells.

Our data show that cellular and humoral immunity against *G. duodenalis* in calves is only induced after several months of infection. Finally protective immunity develops, possibly mediated by IL-17 production.

RELAPSE AFTER TREATMENT WITH MILTEFOSINE FOR VISCERAL LEISHMANIASIS IS ASSOCIATED WITH INCREASED INFECTIVITY OF THE INFECTING *LEISHMANIA DONOVANI* STRAIN.

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Leishmania is an intracellular protozoan parasite that causes leishmaniasis, which can range from a self-healing cutaneous disease to fatal visceral disease depending on the infecting species. Miltefosine is currently the latest and only oral anti-leishmanial that came out of drug discovery pipelines in the past few decades, but recent reports indicate a significant decline in its efficacy against visceral leishmaniasis (also known as kala-azar) in the Indian subcontinent. This relapse rate of up to 20% within 12 months after treatment was shown not to be related to re-infection, drug quality, drug exposure or drug-resistant parasites. We therefore aimed to assess other phenotypes of the parasite that may affect treatment outcome, and found a significant association between the number of metacyclic parasites, parasite infectivity and patient treatment outcome in the Indian subcontinent. Together with previous studies on resistance of *L. donovani* against pentavalent antimonials, these data suggest that infectivity of the parasite, or related phenotypes, might be a more determinant factor for treatment failure in visceral leishmaniasis than drug susceptibility, warranting a re-assessment of our current view on treatment failure and drug resistance in leishmaniasis and beyond.

TRANSMISSION ASPECTS OF FASCIOLOSIS IN VIETNAM

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Fasciolosis is a relatively serious problem and is distributed in many different provinces with a particularly high impact in central Vietnam. However, prevention of fasciolosis might be difficult due to the lack of information about transmission aspects, especially on snail intermediate hosts. In the present study, the objective was to determine the current status of transmission aspects in fasciolosis epidemiology and to provide updated information for the development of control strategies. Lymnaeid snail sampling was conducted in north and central parts in 2012. A questionnaire was developed and used during interviews involving local people and dealing with their eating habits. Snails were identified based on morphology and molecular analysis and were examined regarding to their infection status through microscopy and multiplex PCR. Three snail species were identified as *Austropeplea viridis, Radix rubiginosa,* and *R. auricularia.* Among them, it was the first time *R. rubiginosa* was recorded in Vietnam. *A. viridis* appears to be the most abundant species with diverse distribution in Vietnam. Only *A. viridis* harboured *Fasciola* spp. with an infection rate of 1.17% (299/25,422) as assessed by microscopy. Multiplex PCR was a more sensitive method to detect *Fasciola* spp. in snail when compared to microscopy. *Fasciola* spp. infection rate in *A. viridis* was significantly less by microscopy (1.14%; 57/5,000) than by multiplex PCR (1.82%; 91/5,000) (Chi2 = 7.93; 1df, *P*=0.005). The prevalence of *Fasciola* spp. in snails was significantly higher in central part (1.52%; 167/11,011) than in northern part (0.91%; 132/14,411) (Chi2 = 19.38; 1df; *P*<0.0001). *Fasciola* spp. infection rate was remarkably high in two periods linked to rice cultivation in north while it can take place through the year in central part. Consumption of raw (or undercooked) fresh

vegetable or contaminated drinking water was identified as the main risk factors responsible for transmission of human fasciolosis in Vietnam.

THE IMPACT OF FOREST CONVERSION ON TICK POPULATIONS

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By altering tree species composition and vertical structure, forest management may have a considerable impact on the suitability of forests for ticks and their hosts, thus influencing the dynamics of tick-borne diseases. This is particularly important in the context of ongoing projects that aim to convert pine plantations into semi-natural forests to optimize their ecological and social/recreational functions.
The impact of forest conversion on *Ixodes ricinus* populations was investigated in the Campine region (BE). Two observational studies – a large-scale survey and a local longitudinal study – were carried out in forest stands varying in tree species (pine *vs*. oak) and shrub cover to investigate the effect of landscape and local habitat on tick abundance. Also, a moderate thinning and shrub removal experiment were carried out to verify the influence of vertical forest structure on ticks. Finally, we studied the effect of a seed-addition experiment and an oak mast year on the abundances of rodents and their tick parasites to assess the importance of abundant food resources.
At the landscape level, a positive effect was found for forest edge length (i.e. fragmentation) on the abundance of nymphs and adults. At the local level, the abundances of larvae, nymphs and adults were higher in oak stands compared to pine stands, and increased with increasing shrub cover. Thinning had no effect on tick abundances, while shrub clearing had an adverse effect on the abundances of all life stages up to two years post-clearing. Finally, rodent abundance and the abundance of questing nymphs showed a lagged positive response to acorn mast.
Our results provide more insight into the role of forest composition, forest structure, and landscape configuration on the spatiotemporal variation in tick abundances. We conclude that forest conversion might create suitable habitats for ticks by altering the dynamics between ticks and their key hosts.

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