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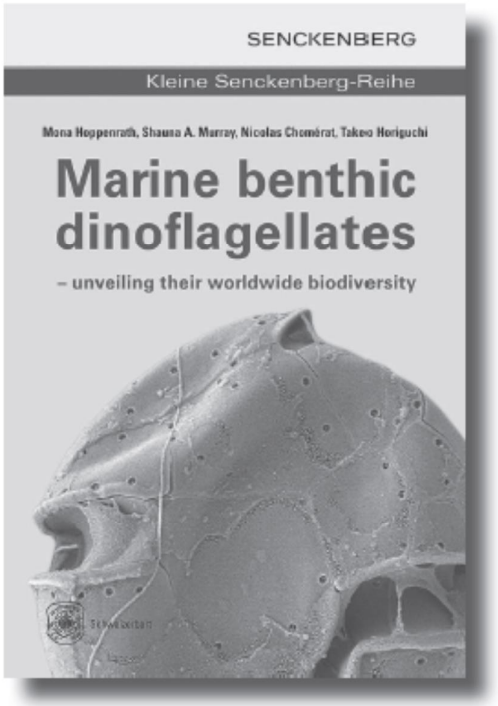

Marine benthic dinoflagellates

– unveiling their worldwide biodiversity

Ed.: Mona Hoppenrath; Shauna A. Murray; Nicolas Chomérat; Takeo Horiguchi

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This publication presents the first summary of our knowledge of benthic dinoflagellate species. Dinoflagellates are important primary producers and symbionts, but, at the same time, also consumers and parasites. Species compositions of benthic habitats are quite distinct from those of planktonic habitats. Less than 10% of the approximately 2000 described extant dinoflagellate species appear to be benthic. They occur in different types of habitats (**chapter II**) and their morphology, their behavior, and some of their life cycles (**chapter VI**) seem to be well adapted to the benthic lifestyle. Information on their geographic distribution is still very limited and is compiled herein (**chapter V**).

The study of harmful benthic dinoflagellates started in the late 1970s when it was suspected that a benthic species, later named *Gambierdiscus toxicus*, was responsible for ciguatera fish poisoning, a type of human poisoning linked to the consumption of certain species of tropical reef fish. As the number of ciguatera fish poisoning incidents increases, and the distribution of toxin producing benthic taxa seems to be expanding, detailed understanding of the species diversity and the ability to accurately identify them is becoming increasingly important (**chapter VII**). Dinoflagellate classification is currently undergoing changes and far from being settled, as new species and genera are discovered and systematic entities are rearranged. Many benthic dinoflagellate genera have unusual morphologies and appear to be only remotely related to known planktonic taxa, so that molecular phylogenetic analyses frequently show little statistical support for any relationship (**chapter IV**).

The book includes the first comprehensive identification help for benthic dinoflagellates. At the same time it aims to lend support in order to improve monitoring efforts worldwide. About 190 species in 45 genera are presented in detail, illustrated with more than 200 color images, approximately 150 scanning electron micrographs, and more than 250 drawings.

E. Schweizerbart'sche Verlagsbuchhandlung
Johannesstr. 3A, 70176 Stuttgart , Germany www.schweizerbart.com
Phone +49 (0)711 351456 0 Fax +49 (0)711 351456 99 order@schweizerbart.de

PROGRAMME

09:30	Registration and coffee	
10:00	BSPP President	Welcome address
Session 1 – Chair: Jean-Claude Dujardin		
10:10	**Keynote:** Prof. Fabienne Tacchini-Cottier (University of Lausanne, Switzerland)	EARLY EVENTS FOLLOWING LEISHMANIA INFECTION
10:50	Annelies Mondelaers (UA)	UNDERSTANDING MIL TREATMENT FAILURE
11:05	Sarah Hendrickx (UA)	RELEVANCE OF INTRACELLULAR PARASITE FITNESS TO ASSESS IN VITRO DRUG RESISTANCE IN LEISHMANIA
11:20	Short Break	
Session 2 – Chair: Dirk de Graaf		
11:30	Linda De Vooght (ITM)	SYMBIOTIC BACTERIA AS A DELIVERY SYSTEM FOR NANOBODIES THAT TARGET THE INSECT-PARASITE INTERPLAY
11:45	Natascha Chavatte (UGent)	FREE-LIVING PROTOZOA ON DISHCLOTHS: OCCURRENCE, DIVERSITY AND POSSIBLE IMPLICATIONS FOR FOOD SAFETY
12:00	Jorgen Ravoet (UGent)	MOLECULAR DIVERGENCE OF HONEY BEE TRYPANOSOMATIDS
12:15	Ellen Lambrecht (UGent)	CYSTS OF FREE-LIVING PROTOZOA: A POTENTIAL VECTOR AND SHELTER FOR FOODBORNE PATHOGENS
12:30	Nele Boon (KUL)	SCHISTOSOMIASIS: THE ROLE OF PARASITE GENETICS IN HUMAN INFECTION AND DISEASE
12:45	Lunch	
13:30	BSPP General Meeting	
Session 3 – Chair: Thomas Geurden		
14:00	**Keynote:** Dr. Nicole Viaene (Institute for Agricultural and Fisheries Research, Belgium)	PLANT PARASITIC NEMATODES: HOW DO WE MANAGE THEM?
14:40	Sien Verschaeve (UGent)	MEASURING LARVAL CONTAMINATION ON CATTLE PASTURES: COMPARISON OF TWO DIFFERENT SAMPLING METHODS
14:55	Ashenafi Kure (Jimma University, Ethiopia)	COMPARISON OF INDIVIDUAL AND POOLED STOOL SAMPLES FOR THE ASSESSMENT OF SCHISTOSOMA MANSONI AND SOIL-TRANSMITTED HELMINTHS INFECTION USING KATO-KATZ TECHNIQUES IN SOUTH-WEST ETHIOPIA.
15:10	Yannick Caron (ULg)	NEW INSIGHT IN LYMNAEID SNAILS (MOLLUSCA, GASTROPODA) AS INTERMEDIATE HOSTS OF FASCIOLA HEPATICA
15:25	Bruno Leveque (UGent)	PARADESIGN: TOWARDS AN ONLINE TOOL TO DESIGN SURVEYS FOR MONITORING MASS DRUG ADMINISTRATION PROGRAMMES IMPLEMENTED TO CONTROL SOIL-TRANSMITTED HELMINTHIASIS IN PUBLIC HEALTH.
15:40	Fransisco J. Morales (VUB)	TOWARDS IMPROVING THE DIAGNOSIS OF HUMAN TOXOCARIASIS THROUGH NANOBODY® TECHNOLOGY
15:55	Coffee break	
Session 4 – Chair: Jan Van Den Abbeele		
16:20	Jennifer Cnops (VUB)	IFN-GAMMA IS CRUCIAL FOR THE INDUCTION OF ACUTE INFLAMMATION AND ASSOCIATED ANEMIA OCCURRING DURING TRYPANOSOMA BRUCEI INFECTION
16:35	Carl De Trez (VUB)	AFRICAN TRYPANOSOMIASIS-INDUCED ALTERATION OF ANTIGEN-SPECIFIC B CELL ANTIBODY TITERS IMPAIRS THE DEVELOPMENT OF COLLAGEN-INDUCED ARTHRITIS IN DBA/1 PRONE MICE AS WELL AS T CELL-DEPENDENT AND – INDEPENDENT HUMORAL RESPONSES
16:50	Eliane Tihon (ITM)	GENETIC DIVERSITY IN THE NATURAL TRYPANOSOMA CONGOLENSE POPULATION
17:05	Irina Matetovici (ITM)	TRYPANOSOME-ASSOCIATED CHANGES IN THE TSETSE FLY SALIVARY GLAND ENVIRONMENT
17:20	Dafra Pharma Best Presentation Award & Zoetis Travel Grant BSPP Logo Contest	
17:45	Reception	

INVITED SPEAKERS

EARLY EVENTS FOLLOWING *LEISHMANIA* INFECTION.

Fabienne Tacchini-Cottier and Benjamin Hurrell
Department of Biochemistry, WHO-Immunology Research and Training Center, Faculty of Biology and Medicine, University of Lausanne, 1066 Epalinges, Switzerland. Fabienne.Tacchini-Cottier@unil.ch

Leishmaniasis is caused by protozoan parasites of the genus *Leishmania*. The infectious flagellated form of the parasites (promastigotes) is inoculated during the insect blood meal. Once inside macrophages, its major host cell where it replicates, it looses its flagella and transforms in its amastigote form. Infection with different *Leishmania* species leads to a wide spectra of diseases ranging from localized, diffuse, cutaneous lesions to mucocutaneous lesions as well as a visceral form of the disease which is fatal if untreated. Leishmaniasis is a neglected disease but WHO estimates that 350 millions people are at risk and there are an estimate of 1 to 1.5 millions cases of cutaneous forms of the disease and 0.5 million of the visceral forms per year. There is currently no efficient vaccine available and resistance to the common drugs is increasing. There is a need to better understand the cellular mechanisms leading to protection.
Neutrophils are massively and rapidly recruited to sites of *Leishmania* infection. They are the predominantly infected cells at the early stages post infection. Growing evidence suggests that these cells play a role in the subsequent development of a *Leishmania*-specific protective immune response. To illustrate the importance of neutrophils during infection, we used the experimental model of *L. mexicana* infection.
Following infection with *Leishmania mexicana*, a species found in the Americas, both humans and mice develop small chronic non-healing cutaneous lesions with persistent parasites, a process linked to the failure to develop a protective immune response. We show here that Neutrophils, not only sequestered most of the parasites, but also formed Neutrophil Extracellular Traps (NETs) at that time, trapping the parasites. In addition, monocyte and monocyte-derived DC recruitment to the site of infection and migration to the draining lymph nodes was tightly linked to the presence of neutrophils as both processes were significantly increased in their absence. Overall, the results that will be presented demonstrate that during the first days of infection, neutrophils contribute to the immune hypo-responsiveness observed following *L. mexicana* infection, preventing via several pathways the onset of a protective immune response critical to control parasite load.

PLANT-PARASITIC NEMATODES: HOW DO WE MANAGE THEM?

Nicole Viaene
Institute for Agricultural and Fisheries Research, Merelbeke; Faculty of Sciences, UG, Ghent University, nicole.viaene@ilvo.vlaanderen.be

Plant-parasitic nematodes are a small fraction of the phylum Nematoda, but they have a huge impact on man by reducing yields of agricultural crops. These microscopic round worms damage plants by feeding on the plant cell content, by migrating through tissues, and providing entrance for pathogenic fungi and bacteria into the already weakened plant.
As most symptoms of infection by plant-parasitic nematodes e.g. reduced plant vigour, are not specific, these organisms are often not recognized as causal agents of crop loss. A first step in managing plant-parasitic nematodes is adequate detection. This requires appropriate sampling, extraction and identification techniques. The latter are changing rapidly from morphology-based identification by specialists using microscopes, to molecular (DNA)-based methods which do not require such specific training. By making detection methods more accessible and cheaper, diagnostic laboratories are able to analyze more samples. Plant-parasitic nematodes, the “hidden enemy”, will become more visible this way, so that the real management can start.

When plant-parasitic nematodes in seeds, wood, flower bulbs or roots are killed by destroying the (imported) commodity, or by heat-treatment or fumigation; the term control sensu stricto is used. However, when dealing with nematodes in farmer fields, even the most toxic nematicide is not able to kill all plant-parasitic nematodes. Moreover, as most nematicides have been banned (at least in Europe) in the last two decades, an integrated management approach is the only option. This means keeping population levels of plant-parasitic nematodes below an appropriate damage threshold so that crop production is possible without economic loss.

Several "old" cultural practices have gained interest again, such as crop rotation, but now using resistant cultivars or green manures. New variations on old themes are solarisation, inundation and anaerobic soil disinfestation, but also adding organic matter to soil. Organic amendments give rise to a larger diversity of soil micro- organisms, including antagonists, and make the soil suppressive and/or or the plant less vulnerable. Currently, many studies are trying to unravel the complexity of these mechanisms so that we can understand the processes between soil, plant and nematode. With the answers we hope to steer the process in the right direction: reducing densities of plant-parasitic nematodes in the field. This could be done by manipulating the soil environment (e.g. adding compost, biocontrol organisms, …) or manipulating the plant (e.g. changing genes). The step from lab and greenhouse trial towards the field environment is the most difficult one, but still needs to be taken for many of the mechanisms discovered so far.

Last, but really first, is of avoidance or prevention: keeping plant-parasitic nematodes out of the field, out of a country or a region. International as well as national regulations, including quarantine status, are very important tools here and even though only a small fraction of harmful nematodes are detected this way, having rules in place reduces spread. Ways of spread, together with survival and basic nematode biology have been neglected topics in this era of molecular research. They are, however, the corner stones of an integrated nematode management.

ORAL PRESENTATIONS

UNDERSTANDING MIL TREATMENT FAILURE

Mondelaers A.[1], Hendrickx S.[1], Boulet G.[1], Dujardin J.C.[2], Delputte P.[1], Cos P.[1], Maes L.[1]
[1] Laboratory for Microbiology, Parasitology and Hygiene, UA, Louis.Maes@uantwerpen.be; [2] Molecular Parasitology Unit, Department of Biomedical Sciences, ITM- jcdujardin@itg.be

Oral miltefosine (MIL) has been introduced as first-line therapy for visceral leishmaniasis in endemic areas with antimonial resistance. Although increased relapse rates were recently reported, MIL-resistant *Leishmania donovani* strains have yet not been isolated. To study MIL-resistance mechanisms and dynamics, we recently developed an *in vitro* procedure to experimentally induce resistance in intracellular amastigotes. Adopting *in vitro* selection on several *L. donovani* and *L. infantum* isolates, only *L. infantum* LEM3323 developed resistance. While the IC_{50}-values of the other strains did not increase, promastigote back-transformation nevertheless became positive at increasing MIL concentrations. These results trigger three research questions: 1/ What relevance does the promastigote back-transformation have to assess drug susceptibility? 2/ What parasitological characteristics form the basis for LEM3323 resistance development? 3/ Is there a difference between induced resistance of LEM3323 compared to *L. infantum* LEM5159 that acquired resistance in the field?
Isolates from cured and relapse patients were subjected to IC_{50} evaluation and promastigote back-transformation. All isolates proved to be MIL-susceptible, while back-transformations were positive at high MIL-concentrations, suggesting reduced susceptibility. In depth evaluation of the assay revealed that a few susceptible parasites may remain viable after MIL treatment giving rise to positive back-transformations. Since treatment failure cannot be linked to *in vitro* resistance, other parasitological, host and/or drug factors should be taken into consideration. For example, in comparison to strains that did not develop MIL-resistance *in vitro*, LEM3323 showed a significantly higher intracellular replication. The infectious and virulent potential of this strain is currently being further investigated. To unravel mechanisms underlying the resistant phenotype, LEM3323 and LEM5159 were subjected to genomic and functional analysis. For the LdMT gene, LEM3323 carries a deletion while LEM5159 shows a non-synonymous SNP. DsRed-transfected LEM3323 and LEM5159 did not accumulate BODIPY-labelled MIL while the susceptible LEM3323 counterpart showed MIL-uptake, endorsing the functional role of LdMT.

RELEVANCE OF INTRACELLULAR PARASITE FITNESS TO ASSESS IN VITRO DRUG RESISTANCE IN LEISHMANIA

Hendrickx S., [1] Mondelaers A., [1] Boulet G., [1] Dujardin JC., [2] Delputte P., [1] Cos P., [1] Maes L.[1]
[1] Laboratory for microbiology, parasitology and hygiene (LMPH), UA; [2] ITM; Louis.maes@uantwerpen.be

The emergence of antimony (Sb^V) resistance in endemic regions with visceral leishmaniasis has shifted therapy towards alternative drugs like miltefosine (MIL) and paromomycin (PMM). However, both drugs may certainly become prone to emergence and spread of primary resistance too. By using a novel *in vitro* resistance selection protocol on intracellular amastigotes, selection for PMM-resistance resulted in rapid generation of several R-strains, while the selection protocol for MIL-resistance only generated one strain (*L. infantum*) with a clearly resistant phenotypeThe primary aim of this study was to evaluate if a shift in parasite fitness occurs in the selected resistant strains compared to the susceptible parent strains. For Sb^V-resistant *L. donovani,* it has been suggested that the acquisition of primary resistance can be accompanied by an evolutionary benefit with increased virulence and spreading potential. In our experimental approach focus was mainly placed on the intracellular amastigote. However, as promastigotes are needed to generate amastigotes, promastigote growth, metacyclogenesis and infectivity were mapped carefully to permit direct comparison between non-selected and selected strains. Upon infection with promastigotes, the intracellular amastigote replication patterns of different *L. donovani* and *L. infantum* lab- and field strains revealed that almost no intracellular replication occurs, leading even to a time-dependent

decrease in amastigote burden. However, the single exception to that pattern was observed in the *L. infantum* strain in which definite MIL-resistance could be obtained, endorsing the importance of "fitness'' parameters in the overall assessment of drug resistance and *in vitro* resistance selection protocols.
To our knowledge, this is the first study that extensively compared intracellular growth characteristics of a panel of different *Leishmania* species and paired drug-susceptible and drug-resistant strains. These results also motivate for continued research on host- and stage-dependent factors influencing intracellular survival and replication.

SYMBIOTIC BACTERIA AS A DELIVERY SYSTEM FOR NANOBODIES THAT TARGET THE INSECT-PARASITE INTERPLAY

De Vooght L.[1], Caljon G.[1,2,3], Hussain S.[2,3] and Van Den Abbeele J.[1,4]
[1] Department of Biomedical Sciences, Unit of Veterinary Protozoology, ITM; [2] Unit of Cellular and Molecular Immunology, VUB; [3] Laboratory of Myeloid Cell Immunology, VIB; [4] Department of Physiology, Laboratory of Zoophysiology, UG

Sodalis glossinidius, a vertically transmitted microbial symbiont of the tsetse fly (*Glossina* sp.), is currently considered as a potential delivery system for anti-trypanosomal components that reduce or eliminate the capability of the tsetse fly vector to transmit parasitic trypanosomes, an approach also known as paratransgenesis. Recently, we developed a plasmid-based expression system allowing *Sodalis* to constitutively express and release functional Nanobodies (Nbs) targeting the *Trypanosoma brucei* VSG surface coat. This was shown in *in vitro* culture conditions as well as *in vivo* in different tissues of the tsetse fly. To improve the stability and expression levels of the anti-trypanosome Nbs, we developed a Tn7-based transposition system for the integration of transgenes into a highly active region of the *Sodalis* genome. Here, genomic integration of a *gfp* reporter gene in *Sodalis* was shown to result in a two-fold increase of the GFP-expression levels compared to the plasmid-based expression system.
Another crucial step in developing paratransgenic tsetse is the sustainable colonization of the fly and its subsequent generations with the genetically modified *Sodalis*. Using a chromosomally GFP-tagged *Sodalis* (rec*Sodalis*) strain, we demonstrated that intralarval microinjection of rec*Sodalis* proves to be essential to achieve vertical transfer and subsequent establishment of rec*Sodalis* into the following generations of progeny.
Finally, since the ingested trypanosome bloodstream forms undergo rapid transformation into the procyclic stage in the tsetse fly midgut, Nbs that target the surface coat of the established insect-stage procyclic trypanosomes will have a longer time frame to interfere with parasite development in the fly alimentary tract. For this, an anti-procyclic *T. brucei* Nb library was generated from which a series of different Nbs were selected that i) bind to the parasite surface and/or ii) compromise the growth of these trypanosomes. Rec*Sodalis* strains expressing anti-procyclics Nbs will now be tested for their ability to block *T. brucei* development in the tsetse fly midgut.

FREE-LIVING PROTOZOA ON DISHCLOTHS: OCCURRENCE, DIVERSITY AND POSSIBLE IMPLICATIONS FOR FOOD SAFETY

Chavatte N.,[1*] Baré J.,[1] Lambrecht E.,[1] Van Damme I.,[1] Vaerewijck M.,[1] Sabbe K.,[2] Houf K.,[1]
[1] Department of Veterinary Public Health and Food Safety, Faculty of Veterinary Medicine, UG; [2] Department of Biology, Faculty of Sciences, UG. [*]*natascha.chavatte@UGent.be*

Free-living protozoa (FLP) play an important role in the ecology and epidemiology of foodborne bacterial pathogens. Dishcloths form a potentially important source of cross-contamination with FLP and foodborne pathogens in food-related environments. At present, no information on the presence of FLP on dishcloths, and the simultaneous occurrence of foodborne pathogens is available. To date no standardized protocols for recovering and quantifying FLP from dishcloths are accessible.

Sampling protocols for the recovery and quantification of FLP from dishcloths were developed and evaluated. These protocols were applied to assess FLP occurrence and diversity on used dishcloths. In parallel, the presence and concentrations of important foodborne pathogens was investigated. Further, the possible impact of various factors on the FLP presence and abundance, and bacterial load was examined.
FLP were found on 89% of the examined dishcloths; 100% of these tested positive for amoebae, 71% for flagellates and 47% for ciliates. The total number of FLP in used dishcloths ranged from 10 to 10^4 MPN/cm^2. Diversity was dominated by amoebae belonging to vahlkampfiids, vannellids, *Acanthamoeba* spp., *Hyperamoeba* sp. and *Vermamoeba vermiformis.* The ciliate genus *Colpoda* was especially abundant on dishcloths while heterotrophic nanoflagellates mainly belonged to the genus *Bodo*, the glissomonads and cercomonads. Detergent use was identified as a prime determinant of FLP concentrations on used dishcloths. Bacterial load on dishcloths was high, with a mean total of aerobic bacteria of 7.47 $\log_{10}$ cfu/cm^2. *Escherichia coli* was detected in 68% of the used dishcloths. Foodborne pathogens including *Staphylococcus aureus*, *Arcobacter butzleri* and *Salmonella enterica* subsp. *enterica* ser. Halle were also recovered.
This study showed that FLP, including some opportunistic pathogens, are a common and diverse group on dishcloths. Important foodborne pathogens are also regularly recovered. This simultaneous occurrence makes dishcloths a potential risk factor for cross-contamination and a microbial niche for bacteria – FLP interactions.

MOLECULAR DIVERGENCE OF HONEY BEE TRYPANOSOMATIDS

Ravoet J.[1], Schwarz R.[2], Evans J.D.[2], de Graaf, D.C.[1]
[1] Laboratory of Molecular Entomology and bee pathology, UG, Jorgen.Ravoet@Ugent.be; [2] Bee Research Laboratory, Beltsville Agricultural Research Center, Beltsville, USA

Trypanosomatids infecting honey bees received only recently attention. After the description of *Crithidia mellificae*, it took almost fifty years until molecular data of markers became available. This led soon to the description of a new trypanosomatid species, *Leptomonas passim*. Research on the related bumble bee parasites *Crithidia bombi* and *Crithidia expoeki* revealed a fragment length polymorphism in the internal transcribed spacer 1 (ITS1), which enabled species discrimination without sequencing. To investigate if this marker can be used in honey bee trypanosomatids, we studied cell cultures of *C. mellificae* and *L. passim* besides infected honey bee samples. As expect, the ITS1 region of both types also contained a fragment length polymorphism. This revealed that L. passim is the dominant species in all investigated countries. We found *C. mellificae* only rarely in Belgian honey bee samples, but surprisingly also in the solitary bee *Osmia cornuta*. Moreover, we were able to amply almost the complete 18S rRNA and ITS1-2 regions of infected honey bees. Other genes like the glycoprotein 63 and cytochrome *b* of one Belgian sample were further compared with the genome of *L. passim* strain SF. Phylogenetic analysis exposed that honey bee trypanosomatids were more related to each other than to those of bumble bees.

CYSTS OF FREE-LIVING PROTOZOA: A POTENTIAL VECTOR AND SHELTER FOR FOODBORNE PATHOGENS

Lambrecht E.[1], Baré J.[1], Chavatte N.[1], Sabbe K.2, Houf K.[1]
[1] Department of Veterinary Public Health and Food Safety, Faculty of Veterinary Medicine, UG; [2] Department of Biology, Faculty of Sciences, UG

The production of resistant, dormant cysts forms an integral part of the life cycle of many free-living protozoa, allowing these organisms to survive adverse environmental conditions. There is increasing evidence that some bacteria escape digestion by bacterivorous protozoa and can survive and even multiply inside active protozoan cells. In some cases, intracystic survival has also been demonstrated, which is especially relevant as cysts may confer high resistance to unfavorable environments. Given

the prevalence of free-living protozoa in food-related environments, it has been hypothesized that these organisms may play an important role in the transmission and epidemiology of foodborne pathogenic bacteria.
The present study investigated the survival capacities of different foodborne pathogens inside cysts of the model protozoon *Acanthamoeba castellanii*. Therefore, invasion assays, encystment monitoring assays and intracystic (stress-)survival assays were performed.
Results indicate that important foodborne bacteria (*i.e*. *Salmonella enterica*, *Yersinia enterocolitica*, *Escherichia coli* and *Listeria monocytogenes)* can survive inside cysts of the ubiquitous amoeba *Acanthamoeba castellanii* and resume active growth after excystment, even when they have been exposed to e.g. antibiotic treatment and highly acidic conditions (pH 0.2). Strain- and species-specific differences in survival period were observed, with *Salmonella enterica* surviving up to three weeks inside the amoebal cysts. These differences were not related to variation in trophozoite invasion/uptake efficiency. Transmission electron microscopy revealed that up to 53% of the cysts were infected with pathogenic bacteria, which were located in the cyst cytosol. Apparently intact cells of another common bacterial pathogen, *Campylobacter jejuni*, were observed inside *A. castellanii* cysts, but no cells were observed after excystment.
The present study indicates that long-term survival of foodborne pathogens in protozoan cysts is possible. This has an impact on the ecology and epidemiology of pathogenic bacteria, as cysts may act as a vector and shelter against hash environmental conditions.

SCHISTOSOMIASIS: THE ROLE OF PARASITE GENETICS IN HUMAN INFECTION AND DISEASE

N. Boon[1,2], F. Van den Broeck[1,2], L. Meurs[2], F. A.M. Volckaert[1], K. Polman[2] & T. Huyse[1,2]
[1] Laboratory of Biodiversity and Evolutionary Genomics, Biology, UL; [2] Unit of Medical Helminthology, ITM

Schistosomiasis is a major, poverty-related disease affecting more than 200 million people in developing countries. It has a complex epidemiology with a large variation in infection intensity and schistosome-related pathology. It is still not fully understood why certain people living in the same endemic area are heavily infected and develop disease while others do not. It has been shown that factors such as host genetic background and water contact play an important role, but a large part of the variation in infection intensity and pathology in humans remains unexplained.
To untangle the importance of these factors, knowledge about the influence of parasite genetics on host's disease patterns is fundamental. We conducted a large epidemiological study in northern Senegal. We genotyped 1692 *S. mansoni* larvae collected from 45 human hosts with nine microsatellite loci and linked this with host data such as age, gender, infection intensity, liver and bladder morbidity. We found a positive relationship between schistosome infection intensity (measured as eggs per gram feces (epg)), and the frequency of a certain parasite allele. We corrected for age, sex, and co-infection with *S. haematobium*. This trend is found with linear regression and redundancy analysis. Two other alleles in this locus were negatively correlated with epg. If we divide infection intensity by worm burden (measured by parasite antigen levels in the blood serum of patients), we have a measure of parasite fecundity. This parameter appeared also positively correlated with the specific allele. This microsatellite locus is located in the untranslated region (UTR) of a protein kinase gene. Inhibiting this gene in adult schistosomes resulted in declined egg production by 30%, and reduced muscle contraction.

MEASURING LARVAL CONTAMINATION ON CATTLE PASTURES: COMPARISON OF TWO DIFFERENT SAMPLING METHODS

Verschave S.H.[1*], Levecke B.[1], Duchateau L.[2], Vercruysse J.[1], Charlier J.[1]
[1] Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, UG, sien.verschave@ugent.be;
[2] Department of Physiology and Biometrics, Faculty of Veterinary Medicine, UG

Assessing levels of parasitic nematode contamination on cattle pastures is of great value in epidemiological studies. However, direct quantification of the infective L_3 on herbage is time consuming. Recent research improved the repeatability of the sample processing and simplified nematode species identification by molecular characterisation. However, the herbage collection is not standardized and remains labor intensive. Few studies are available on variation induced by different sampling methods, therefore, the aim of this study was (1) to compare pasture larval counts and time to sample for 2 different sampling methods and (2) to assess the amount of variation in larval counts at the level of sample plot, pasture or season, respectively. Eight young stock pastures were sampled in 3 different seasons. On each pasture, herbage samples were collected through a double-crossed W-shape (method 1) and random located plots (method 2). For each method, time to sample was also recorded. The average (±standard deviation (SD)) pasture contamination found using sampling method 1 and 2 was 325 (±479) and 305 (±444) L_3/kg dry herbage, respectively. The highest count for both methods was seen in autumn on the same pasture (1854 and 2090 L_3/kg dry herbage using method 1 and 2, respectively). Discrepancies in larval counts for samples of the same pasture and season were often seen, but no significant difference (P=0.38) in counts between methods was found. When pasture surface area was larger (>1 ha), method 2 was less time consuming than method 1. Because time to sample for method 2 was less, this method was further assessed. The variation (variance coefficient (v.c.)) in larval counts was only poorly explained by season (v.c.=0.15) or pasture (v.c.=0.55), indicating a strongly aggregated distribution of L_3 on pasture. Using the observed aggregated distribution, the required number of plots to sample in order to obtain a useful estimation of the pasture contamination was calculated.

COMPARISON OF INDIVIDUAL AND POOLED STOOL SAMPLES FOR THE ASSESSMENT OF SCHISTOSOMA MANSONI AND SOIL-TRANSMITTED HELMINTHS INFECTION USING KATO-KATZ TECHNIQUES IN SOUTH-WEST ETHIOPIA.

Kure Ashenafi[1], Mekonnen Zeleke[1], Dana Daniel[1], Bajiro Mitiku[1], Vercruysse Jozef[2], Levecke Bruno[2]
[1]Department of Medical Laboratory Sciences and Pathology, College of Public Health and Medical Sciences, Jimma University, Ethiopia; [2]Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, UG

Our group has recently shown that pooling of stool samples allows rapid assessment of intensity of soil-transmitted helminth (STH, *Ascaris lumbricoides*, *Trichuris trichiura* and hookworm) infection intensity using the McMaster egg counting method. In the present study we evaluated our pooling strategy for the assessment of STH and *Schistosoma mansoni* infections. In addition, we compared the time between an individual and pooled examination strategy. A cross-sectional survey was conducted in 360 school children 5 to 18 years of age from six schools in Jimma Zone. In both individual and pooled samples (pools sizes of 5, 10 and 20) fecal egg counts (FEC) by means of eggs per gram of stool (EPG) were determined using the Kato-Katz thick smear. Except for hookworms, there was a significant correlation between the mean of the individual FECs and the FECs of the pooled samples for *A. lumbricoides*, *T. trichiura* and *S. mansoni*, regardless of the pool size. Mean FEC were 2,596 EPG, 125 EPG, 47 EPG, and 41 EPG, for *A. lumbricoides*, *T. trichiura*, *S. mansoni* and hookworm, respectively. There was no significant difference in infection intensity between the examination of individual and pooled stool samples, except for hookworms. For this STH, pools of 10 resulted in a significant underestimation of infection intensity. The total time to obtain individual FECs was 65 h 5 min. For pooled FECs, this was 19 h 12 for pools of 5, 14 h 39 min for pools of 10 and 12 h 42 min for pools of 20. The results indicate that pooling of stool sample also holds promise as a

rapid assessment of infections intensity for Kato-Katz thick smear and *S. mansoni*. In this setting, the time in the laboratory can be reduced with 70% when pools of 5 instead of individual stool samples are screened.

NEW INSIGHT IN LYMNAEID SNAILS (MOLLUSCA, GASTROPODA) AS INTERMEDIATE HOSTS OF *FASCIOLA HEPATICA* (TREMATODA, DIGENEA) IN ECUADOR.

Y. Caron[1*], M. Celi-Erazo[2], C. Saegerman [3], B. Losson[1], W. Benítez-Ortíz[2, 4]
[1]Research Unit in Parasitology and Parasitic Diseases, Fundamental and Applied Research for Animals & Health (FARAH), Faculty of Veterinary Medicine, ULg; [2]International Center for Zoonosis (ICZ), Central University of Ecuador, Quito, Ecuador
[3]Research Unit of Epidemiology and Risk analysis applied to Veterinary Sciences, FARAH, Faculty of Veterinary Medicine, ULg; [4]Veterinary Medicine and Zootechny Faculty, Central University of Ecuador, Quito, Ecuador; [*] ycaron@ulg.ac.be

Fasciolosis is a widely distributed disease in livestock in South America but knowledge about the epidemiology and the intermediate hosts are scarce in Ecuador. During 3 months, lymnaeid snails were sampled (n=1482) in Machachi province in two biotopes located in a highly endemic area. The snails were identified (based on morphology and ITS2 sequences) and the infection status was established through microscopic dissection and a multiplex PCR-based technique. If morphologic-based techniques were not useful to accurately named the one species collected, phylogenetic study ascribed it to *Galba* sp. (= *L. schirazensis* = *G. cryptica*). Rediae were observed in 1.75 % (26/1482) and *Fasciola* sp. DNA was detected in 6% (89/1482) of the collected snails. The relative sensitivity and specificity of the microscope related to the PCR results was 25.84% and 99.78% respectively. The mean size of the snails recorded positive for *F. hepatica* through crushing and microscopy was significantly higher than the mean size of negative snails. There was not such difference in PCR positive snails. The role of *Galba* sp. as an intermediate host of *F. hepatica* in Ecuador is discussed and a hypothesis of an adaptation of the snail to the trematoda is formulated. For the first time, an epidemiological survey, based on molecular biology-based techniques assessed the role of lymnaeid snail in the epidemiology of fasciolosis in Ecuador.

PARADESIGN: TOWARDS AN ONLINE TOOL TO DESIGN SURVEYS FOR MONITORING MASS DRUG ADMINISTRATION PROGRAMMES IMPLEMENTED TO CONTROL SOIL-TRANSMITTED HELMINTHIASIS IN PUBLIC HEALTH.

Levecke B.[1], Anderson R.M.[2], Berkvens D.[3], Charlier J.[1], Devleesschauwer B.[1,4], Speybroeck N.[4], Vercruysse J.[1], Van Aelst S.[5,6]
[1]Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, UG; [2]Department of Infectious Disease Epidemiology, Faculty of Medicine, Imperial College London, London; [3]Department of Biomedical Sciences, ITM; [4]Institute of Health and Society (IRSS), Faculty of Public Health, UCL; [5]Department of Applied Mathematics, Computer Science and Statistics, Faculty of Sciences, UG; [6]Department of Mathematics, Faculty of Sciences, KUL

Roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*) and hookworms (*Ancylostoma duodenale* and *Necator americanus*) infect millions of children in sub-tropical and tropical countries, resulting in malnutrition, growth stunting, intellectual retardation, and cognitive deficits. To fight these worms, large-scale deworming programmes are implemented in which anthelmintic drugs are administered. This world wide upscale of deworming programmes creates the need for a monitoring system that allows programme managers, policy-makers and donors of the drugs to assess whether the objectives are being met and, if necessary, to adjust the implemented strategy. Thus, it will be imperative to periodically assess worm infections by means of prevalence and infection intensity to determine whether the deworming programme progresses as anticipated. We developed a mathematical framework based on worm egg counts in stool allowing health-care decision makers to adapt their survey design according to both local worm epidemiology (level of aggregation and intensity of worm infections) and resources. To bridge the gap between this mathematical framework and the end-users we developed ParaDesign, an online interface that guides the user in designing an appropriate survey without the need of prior mathematical or statistical knowledge. At

the meeting we will briefly outline the underlying mathematical framework. Subsequently, we will demonstrate selective features of the online tool. Finally, we will highlight features that will be covered by ParaDesign in the near future.

TOWARDS IMPROVING THE DIAGNOSIS OF HUMAN TOXOCARIASIS THROUGH NANOBODY® TECHNOLOGY

Morales FJ.[1], Sariego I.[2], Polman K.[3], Muyldermans S.[1]
[1] Laboratory of Cellular and Molecular Immunology, VUB, Francisco.Morales.Yanez@vub.ac.be; [2] Departamento de Parasitologia, Instituto de Medicina Tropical "Pedro Kouri", La Habana, Cuba, idalia@ipk.sld.cu; [3] Department of Biomedical Sciences, ITM; kpolman@itg.be

INTRODUCTION: Human Toxocariasis (HT) is one of the most common helminthiases worldwide. Seroprevalence of the disease ranges from 2% in rural populations of developed countries, up to 90% in tropical areas. Currently, diagnosis of HT relies on serology and clinical data, not allowing to distinguish between current and past infections. We propose a novel approach to detect parasite proteins in blood samples based on the development of specific single variable domain fragments (VHH) of camelids against the excretory secretory proteins (ESP) of *Toxocara canis*.
METHODOLOGY: To construct a VHH library, an alpaca was immunized with *T. canis* ESP and RNA from lymphocytes in peripheral blood used as template to amplify VHH-encoding sequences. The diagnostic test consists of a sandwich ELISA made up of a capturing Nanobody® cloned in pHEN6c vector and a detection Nanobody® biotinylated through site-specific coupling in pHEN25 vector containing a Cys-terminal. Cross-reactivity was tested by sandwich ELISA with excretory antigens of *Ascaris lumbricoides* and *A. suum* as well as with serum from negative samples. RESULTS: A library of $3x10^8$ transformants was set up of which 84% contained a plasmid encoding the VHH sequence. From this gene bank, 20 different nanobodies spread over 10 different groups were identified. The combination 2TCE49/1TCE52 had the best OD signal in sandwich ELISA with no cross-reactivity with other helminthes proteins. The limit of detection using ELISA sandwich format in negative samples spiked-in with ESP was <100 ng. DISCUSSION: Diagnosis of current and past infections is crucial in the control of HT, particularly in endemic areas. Upon these preliminary data, we consider that Nanobody®-based sandwich ELISA represents a promising tool to develop a specific and sensitive test to identify active Toxocara infections. The detection limit needs to be further improved through more sensitive techniques like the Proximity Ligation Assay (PLA).

IFNG IS CRUCIAL FOR THE INDUCTION OF ACUTE INFLAMMATION AND ASSOCIATED ANEMIA OCCURRING DURING *TRYPANOSOMA BRUCEI* INFECTION

J. Cnops[1], B. Stijlemans[2], C. De Trez[1], F. Kauffman[1], J. Keirsse[2], F. Brombacher[3] and S. Magez[1]
[1] VIB, Department of Structural Biology; VIB, Department of Myeloid Cell Immunology; International Centre for Genetic Engineering and Biotechnology, University of Cape Town

During murine trypanosomiasis the onset of inflammation occurs promptly after infection. An enormous influx of inflammatory monocytes and neutrophils occurs in liver and spleen 72 hours post infection, accompanied by a burst of pro-inflammatory cytokines in the serum. Coinciding, acute anemia develops as witnessed by a 50% reduction in red blood cells within 24 hours. Using a new *in vivo* erythrophagocytosis assay, we show that this acute anemia is a result of enhanced erythrophagocytosis by activated liver monocytes and neutrophils.
IFNg, produced immediately after infection by NK(T) and CD8 T cells, is one of the major cytokines driving this acute inflammatory reaction. IFNgR-/- mice display a reduced influx of inflammatory monocytes and neutrophils in the liver and consequently don't suffer from acute anemia. WT mice infused with anti-NK1.1 or anti-CD8 antibodies display the same reduced anemia phenotype as IFNgR-/- mice, confirming their role in IFNg production. In addition, adoptive transfer of CD8 T cells in CD8-/- mice, reconstitutes the anaemic phenotype.

In conclusion these results indicate that NK(T) cells, CD8 T cells and IFNy are crucial for induction of acute inflammation and the associated anemia during Trypanosoma brucei infection.

AFRICAN TRYPANOSOMIASIS-INDUCED ALTERATION OF ANTIGEN-SPECIFIC B CELL ANTIBODY TITERS IMPAIRS THE DEVELOPMENT OF COLLAGEN-INDUCED ARTHRITIS IN DBA/1 PRONE MICE AS WELL AS T CELL-DEPENDENT AND –INDEPENDENT HUMORAL RESPONSES

Carl De Trez[1], Brunette Katsandegwaza[1], Guy Caljon[2] and Stefan Magez[1]
[1] Research Unit of Cellular and Molecular Immunology, VUB; VIB Department of Structural Biology, Brussels, Belgium. [2]Unit of Veterinary Protozoology, Department of Biomedical Sciences, ITM; Unit of Cellular and Molecular Immunology, VUB; Laboratory of Myeloid Cell Immunology, Vlaams Instituut voor Biotechnologie VIB

Trypanosoma brucei parasites are extracellular protozoan hemoflagellates causing African trypanosomosis, also known as potentially fatal sleeping sickness disease in humans and Nagana in livestock in sub-Saharan Africa. The transmission of *T. brucei* between mammal hosts occurred via the bite of infected tsetse flies (genus *Glossina*). Nagana causes three million cattle deaths every year. The associated economic loss in livestock production is estimated at a staggering 4 billion USD/year. The best strategies to avoid this will be vaccination. However vaccination strategies have all been unsuccessful so far. Therefore, understanding the mechanisms underlying this failure has to become a priority. Recently, our group has demonstrated that trypanosome infection induces a rapid decline in homeostatic B cell subsets causing the abolishment of unrelated vaccine-induced protection. However, the impact of *T. brucei* infection on specific circulating antibody titres against unrelated antigens in a physiological and pathologic context has never been investigated. Therefore we focused our attention on B cell-mediated collagen-induced arthritis model. We observed a substantial abolishment in the development of collagen-induced arthritis in *T. brucei*-infected prone mice that correlates with a drastic decrease in serum-associated anti-Type II collagen antibodies of the different IgG isotypes. Treatment of infected mice with Berenil, a trypanolytic drug, restored arthritis development. Using another well-defined model, the thymo-dependent hapten-carrier NP-CGG emulsified in Alum adjuvant, we observed that post-vaccination *T. brucei* infection is abrogating the titres of CGG-specific as well as NP-specific IgG1+ antibodies, which is a hallmark of memory responses in this model, independently of their affinity. The administration of NP coupled to Ficoll, a thymo-independent antigen, followed by the infection with *T. brucei* also induces a decrease of anti-NP IgG3+ antibodies.
Together, these data suggest that *T. brucei* infection is impairing every stages of B cell development, including effector plasma B cells, independently of their specificity or affinity.

GENETIC DIVERSITY IN THE NATURAL TRYPANOSOMA CONGOLENSE POPULATION

Tihon E[1], Imamura H[2], Van den Broeck F [1,2], Dujardin JC[2], Van Den Abbeele J[1]
[1] Unit of Veterinary Protozoology, ITM; [2] Unit of Molecular Parasitology, ITM

Trypanosoma congolense is one of the major parasites responsible for Animal African Trypanosomiasis, a disease affecting about 10 million km2 of the sub-Saharan region and considered as one of the principal causes of hunger and poverty in this region. Despite the pathological impact of the parasite, in depth information on its genetic diversity and population structure and dynamics is highly limited.
In order to explore the genetic diversity of the T. congolense natural population, we selected 54 strains sampled between 1971 and 2010 in 9 different countries of Africa where the disease is endemic. We selected both Savannah-type (50) and Forest-type (4) strains and used a high-throughput sequencing approach (Illumina Miseq) to identify genome-wide SNPs and genetic structure variation. A total of 614859 SNPs were identified in the whole population, revealing a high diversity among samples. When focusing on the Savannah strains only, 411781 SNPs were identified,

of which 251333 appeared in coding regions and 209933 were non-synonymous mutations. Various long deletions in both coding and non-coding regions have been detected in multiple strains, but no changes in ploidy were observed. Ubiquitous chromosomal phylogenic mismatches as well as haplotype pairwise phylogenic mismatches suggest that recombination and complex genetic exchanges are frequent in T. congolense. Additional phylogenic and structure analysis highlighted a geographically asymmetric genetic diversity in T. congolense, underlying an extremely high diversity among the strains collected in Zambia compared to the rest of the samples. Here, the factors that have resulted in this genetic diversity remain speculative but we suggest that the close proximity to wildlife observed in this region is most likely playing a key role.

TRYPANOSOME-ASSOCIATED CHANGES IN THE TSETSE FLY SALIVARY GLAND ENVIRONMENT

Matetovici I.[1], Caljon G.[1], Van Den Abbeele J.[1]
[1]Unit of Veterinary Protozoology, Department of Biomedical Sciences, ITM, imatetovici@itg.be, gcaljon@itg.be, jvdabbeele@itg.be

For their transmission, African trypanosomes rely on their blood feeding insect vector, the tsetse fly (*Glossina* sp.). Here, ingested *T.brucei* parasites (including the two human pathogenic species) have to overcome a series of barriers in the tsetse fly alimentary tract to colonise permanently the salivary glands and differentiate to the final metacyclic, infective stage. Unravelling the molecular interplay between trypanosomes and the tsetse fly is of high interest that could result in alternative ways to minimise or block the parasite transmission. To assess the impact of *T. brucei brucei* infection on the salivary gland environment, we conducted a genome-wide RNA-seq analysis of the gene expression profile of trypanosome-infected salivary glands versus uninfected glands. Out of 907,153,683 raw reads generated, 702,470,322 (77.43%) uniquely aligned to *G. morsitans* genome and for the infected glands approximatively 20% of the total reads mapped to the *Tbb* genome. Transcriptomic responses upon *Tbb*-infection included an up regulation of genes in functional categories like immunity and response to stimulus (such as several genes of the IMD-signalling pathway, serpins, serine proteases, thiolester-containing proteins, antioxidant response), cell adhesion and junction and cytoskeletal rearrangements. The down-regulated transcripts comprised some of the salivary protein genes (e.g. endonucleases, adenosine deaminases, and 5'-nucleotidases). Overall, this study shows that the interaction between the parasite and salivary gland environment is complex with many different pathways and processes being affected. These first results lay the basis for more detailed work on the parasite-host molecular interactions in the tsetse fly salivary glands.

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